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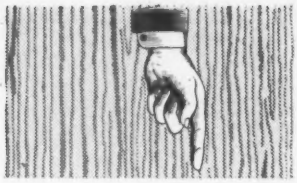

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THE STRENGTH LATENCY RELATION OF AXONS

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IT IS well known since the work of Blair and Erlanger ([33]), that the time interval which elapses between the application of a stimulus to a nerve fiber and the appearance of its action potential, is such that it varies greatly if the stimulus is of a just threshold intensity; it is also known from the same work, that the interval becomes progressively shorter as the stimulus intensity is raised up to twice its threshold value. At that time, the effect of greater stimulus intensities could not be determined on account of limitations imposed by the type of preparation used. In the present study some of these technical limitations have been overcome and a further study of the latency of initiation of impulses under different conditions has been undertaken.

METHOD

Unless otherwise stated, the experiments were performed on frog single nerve fibers, at room temperature (approximately 22° C). The sciatic nerve of the frog *(Rana montezumae)* was excised together with the 8th and 9th spinal nerves, and a single nerve fiber was isolated from one of the branches of the tibial nerve. Usually the segment isolated was about 1 mm and no node of Ranvier was exposed. The conduction velocity of the fibers studied, ranged between 30-50 m/s at room temperature. The dissection complefed, the preparation was transfered to a Petri dish containing mineral oil and was placed on platinum wire electrodes. Two of these were used as stimulating electrodes. The portion of nerve underlying the distal stimulating electrode was crushed with a forceps. The stimuli (rectangular pulses) were delivered by two

* This work was made possible by a grant from the United Cerebral Palsy Association to Dr. Arturo Rosenblueth.

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coupled Grass stimulators (Model S4). To isolate the stimulus from ground, Grass stimulus isolation units (SIU4) were used. In general the frequency of stimulation was 12.5 per second.

For the recording of the nerve fiber potentials, one electrode, connected to ground, was placed at the point of emergence of the single fiber from the nerve trunk, and the other was placed under the distal portion of the nerve, which was either crushed or treated with a concentrated solution of KCl, in order to make the records monophasic. The electric responses of the nerve fibers were led to a cathode-follower input stage coupled to a capacity-coupled preamplifier and from this to one of the channels of a dual-beam oscilloscope (Dumont 322). The frequency characteristic of the system was flat up to 4.5 kilocycles and dropped to 50 percent at 43 kilocycles. The responses were photographed on 35 mm film with a Grass kymograph camera and the measurements were made after projection. Under these conditions and with the sweep speeds used, the time resolution was of the order of 5-10 μs.

Since the beginning of the action potential is difficult to determine, the latency was measured from the stimulus artifact to the moment at which the responses attained 50 per cent of their maximal amplitude. The results were similar to those found in a few experiments in which the latency was measured from the beginning of the stimulus artifact to the beginning of the action potentials.

RESULTS

A) *The influence of the intensity of the stimuli upon the latency of initiation of the action potential.* — The most direct method to determine the moment at which impulses are initiated by stimuli of different intensities, would be to record the responses at the site of initiation. This procedure is feasible, but the artifacts introduced by strong stimuli are so large that the responses are greatly distorted, and it is hard to determine their latencies. To obviate this difficulty the impulses were recorded from a point placed at some distance from the stimulating electrode, thus minimizing importantly the stimulus artifact. The time interval between the application of a stimulus and the appearance of the response at the recording site includes then the latency of initiation and the propagation time. It may be assumed, that under the conditions in which these experiments were made the conduction time was practically constant and so the latency measured differs from the true latency of initiation by an amount equal to the conduction time.

The possibility of shifts in the locus of initiation should be considered. If they should occur they would appear as abrupt changes in the latency which should be of about 40 μs, because the impulses would then be initiated at the low-resistance neighboring nodes. Actually, such changes in latency were seen occasionally when strong stimuli were employed. In other cases, with brief intense stimuli, the latency was abruptly reduced almost to zero, producing a sharp break in the strength-latency curve. This change in latency can be attributed to a shift of the site of initiation from the stimulating electrode to the proximal recording lead, probably due to a capacitative coupling between the two electrodes.

Figure 1 shows the results of an experiment in which the conduction distance was 30 mm; the abscissae are the intensities of a pulse of 1 ms duration, in threshold units, and the ordinates indicate the latencies of the corresponding responses. Each point is the average of the latencies of about 7 responses. The figure shows that when the intensity reached about 40 times threshold a constant minimal latency was attained. This

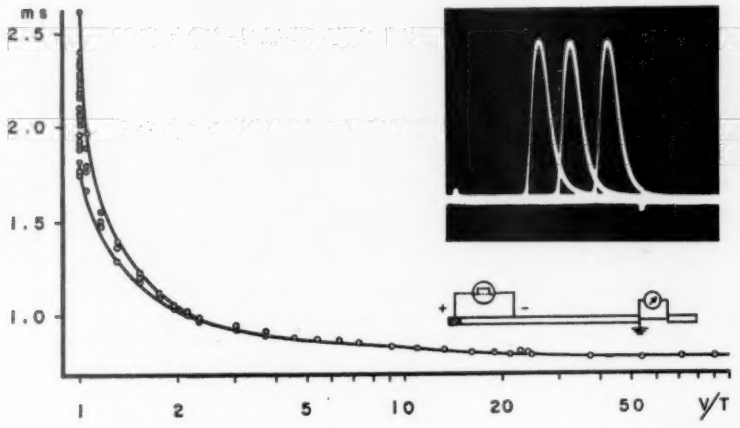

FIG. 1. — *Variations in the latency of initiation of impulses as a function of the strength of a cathodal pulse of 9 ms duration. Records taken as shown in the diagram. Conduction distance: 30 mm. Abscissae: strength of stimuli, in threshold units, logarithmic scale. Ordinates: latency of the responses, measured from the beginning of the stimulus to the moment at which the spike attains half its maximal amplitude.*

The inset shows the latency variation at threshold for another fiber. The duration of the cathodal stimulus (signaled by the two small excursions) was 5 ms. Conduction distance 18 mm. The spikes measure 28 mv in amplitude.

In this and the following figures, unless otherwise stated, the records were taken from isolated frog single fibers.

intensity at which the latency was practically constant varied in different fibers from 30-80 times threshold. In some fibers the total range of intensity studied was from 1 to 210 times threshold without producing any noticeable irreversible effects at the stimulated site.

As the intensity of the stimulus decreases the fluctuations of the latency increase and reach a maximum at threshold (figs. 1, 8, 10, 11). In figure 1 the threshold latency varied from 1.75 to 2.61 ms. The inset in figure 1 shows the threshold play of latency for another fiber; it was obtained by opening the camera shutter for 1 s while the fiber was being stimulated at the rate of 12.5/s with a pulse of 5 ms duration.

In order to determine whether or not the latency at threshold stimulation varies at random, in two experiments the nerve fiber was stimulated at three different frequencies (6, 12.5 and 25 per second) and the

latency of successive responses was measured. No cyclic variations appeared, but the latencies of successive responses varied at random. With threshold stimulation not all the stimuli are effective in eliciting propagated impulses. The failure to evoke responses also occurred at random.

B) *The influence of the duration of the stimuli.* — In these experiments the strength-latency curves were determined for stimuli of different durations. The usual procedure was to alternate stimuli of long and short duration, and to measure for each a complete curve. In some fibers up to twelve different durations were studied. The shortest duration used was 0.01 ms and the longest varied from 10 to 100 ms. In a few experiments, in order to reduce the polarization of the Pt electrodes the stimuli were delivered through Ag-AgCl-agar Ringer electrodes. The results were similar to those obtained with the platinum wires.

Figure 2 illustrates a typical experiment. In order to make the graphs clearer, only the curves corresponding to four different durations are shown. For high voltages the curves coincide. As the voltage is decreased the curves diverge progressively-for a given voltage the latency with a pulse of long duration is smaller than that obtained with a stimulus of shorter duration. The range of latency-play at threshold and the maximal latencies decreased as the durations of the stimuli were made smaller.

The data shown in figure 2 are replotted in figure 3, in threshold units for the abscissae. To simplify, the curve for the 0.1 ms stimulus

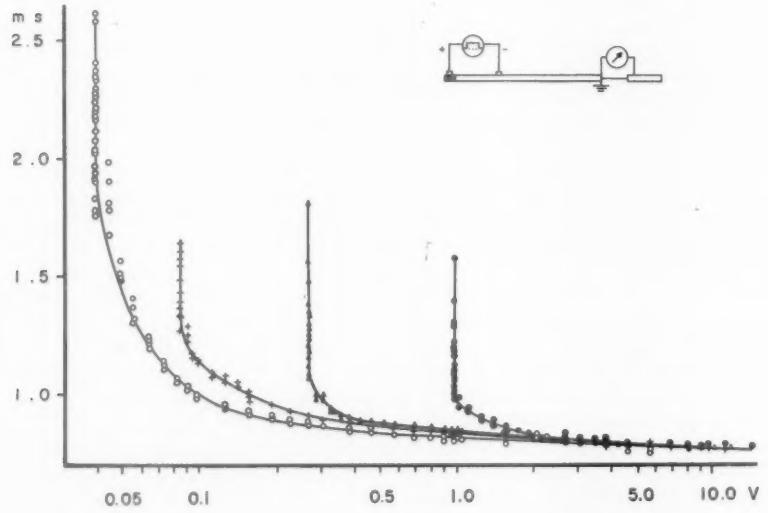

FIG. 2. — *Strength-latency curves for cathodal stimuli of the following durations: 9 ms (circles); 0.4 ms (crosses); 0.1 ms (triangles) and 0.01 ms (dots). Abscissae: intensity of the stimuli in volts (logarithmic scale). Ordinates: latency in milliseconds. The conduction distance was 30 mm.*

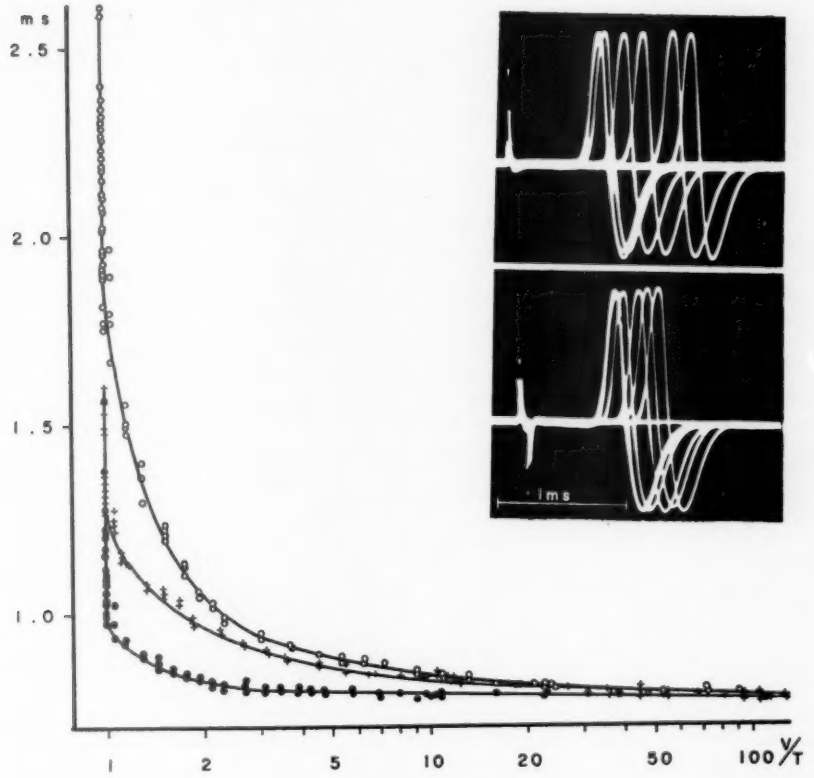

FIG. 3. — *Strength-latency curves for stimuli of different durations, constructed with some of the data shown in figure 2. The durations of the stimuli were: 9 ms (circles); 0.4 ms (crosses) and 0.01 ms (dots). Abscissae: strength of the stimuli in threshold units (logarithmic scale). Ordinates: latency in milliseconds.*

The inset shows records from the same fiber that illustrate the maximal latency variation for stimuli of two different durations: 3.7 ms (upper record) and 0.1 ms (lower record). The threshold for the 3.7 ms pulse was 0.092 v; that for the 0.1 ms pulse was 0.27 v. In both cases superimposed photographs were taken of the responses to stimulus intensities from threshold to 1.08 v.

has not been drawn. Here again, when the stimulus is several times threshold there is no difference between the curves. As the intensity decreases the curves diverge and this divergence increases as the threshold intensity is approached. In contrast with figure 2, in threshold units for the same intensity of the stimulus, the latency is greater for the long than for the brief stimuli.

The records in the inset of figure 3 were obtained 5 hours after the data in figure 2 had been secured. They show the latency variation

for a stimulus of 3.7 ms (upper record) and of 0.1 ms (lower record). In each record 2 superimposed photographs were taken when the stimulus was threshold; the intensity was then progressively raised and the corresponding pictures were again superimposed.

For any particular fiber there is a duration of stimulus beyond which any further increase does not produce any distinguishable effect on the strength-latency curve. This duration varies from one fiber to another; it usually ranged in the fibers studied from 2 to 5 ms.

The maximal range of latency variation for a stimulus of threshold intensity was compared in different fibers by observing the play of latency for pulses of a duration greater than 5 ms. It was found that this play of latency varied from 0.55 to 4.05 ms (1.2 was the mean in 14 experiments).

The maximal increment of latency (greatest latency observed minus the least obtained with high intensities) for stimuli of long durations, was in the fibers studied from 1.0 to 6.0 ms (mean 2.57 ms). This maximum was usually greater in the fibers that gave repetitive firing in response to a stimulus of long duration (see below) than in those that gave single responses. The fibers that had the greatest maximal increment had usually a large threshold play.

C) *The influence of polarizing currents.* — In these experiments the platinum stimulating electrodes were replaced by Ag-AgCl-agar Ringer electrodes, and through this pair of electrodes both the stimulating and polarizing currents were delivered. We will refer to the polarizing current as cathodal or anodal according to whether the cathode or anode was placed at the intact region of nerve. The test rectangular pulses were delivered at the frequency of 12.5 per second.

When an anodal polarizing current was applied to the nerve, the threshold (voltage at which about 75 per cent of the stimuli are effective) increased when the polarizing current was established. This increase was proportional to the intensity of the current and was followed by a slow and small progressive decrease. After the break of the current there occurred a sudden decrease of threshold below the normal level followed by a slow return to the resting excitability. When the polarizing current was cathodal, there occurred at the make a lowering of the threshold followed by a rise. The rate and magnitude of this subsequent rise were proportional to the intensity of the current, and in contrast to the effects of anodal currents the threshold after the make of the current crossed the normal level. In some fibers it took several minutes before the threshold during cathodal polarization had risen over the normal level while in others this was accomplished in about one minute.

Figure 4 shows the results of an experiment in which the latency (ordinates) of the responses evoked by a cathodal pulse of 4 ms duration was measured during anodal polarization with different intensities (abscissae). In the case of the curve drawn through the full circles the intensity of the pulse used was 4.85 while in that of the circles it was 6.47 times threshold. The latency of the responses increases as the intensity of the polarizing current is increased until an intensity is reached at which the latency varies and sometimes the stimuli fail to

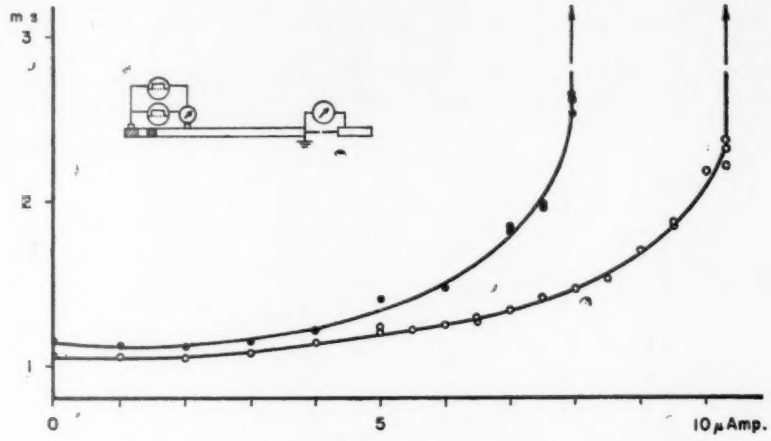

FIG. 4. — *Variations in the latency to a cathodal pulse of 4 ms duration, during the application of anodal polarizing currents of different intensities. The experimental arrangement is shown in the diagram. The fiber was 12 μ in diameter and had one node exposed in the dissected segment. The distance between the stimulating electrodes was 10 mm, and the conduction distance was 31 mm.*

Abscissae: intensity of the polarizing currents. Ordinates: latency in milliseconds. The two curves correspond to two different intensities of the test stimuli: 4.85 and 6.47 threshold, dots and circles, respectively. The arrow in each curve marks the intensity of polarizing current at which the test stimuli failed to elicit spikes.

evoke a response (arrows). When the intensity of the test stimulus is increased, the latency of the responses becomes smaller and the intensity of polarizing current at which the stimulus becomes ineffective is increased (cf. the two curves in fig. 4).

Figure 5 illustrates the results obtained from another fiber in one of the experiments in which instead of varying the intensity of the polarizing current, this was kept constant while the intensity of the test stimulus was changed. In this experiment the strength-latency relation was first determined for a pulse of 0.4 milliseconds (triangles), then, without changing the duration of the pulses, a second determination of the strength-latency relation was made during anodal polarization (3.0 μamp; circles). About 8 minutes later, the threshold had returned to normal; a cathodal polarizing current of the same intensity as the anodal was applied and the data for another strength-latency curve were gathered (dots).

As shown in figure 5, for any stimulating voltage the latency of the responses obtained during anodal polarization is greater than that obtained without polarization. The divergence between the curves is greater at low voltages of stimulation and tends to dissappear at high voltages. The curves taken during cathodal polarization may lie either

at the right or at the left of the normal curve depending on the intensity of the current and the time after the onset of polarization at which the latency determinations are made; in the present case for any stimulating voltage the latency during cathodal polarization was smaller than the normal. The minimal latencies at threshold are practically the same in the three curves.

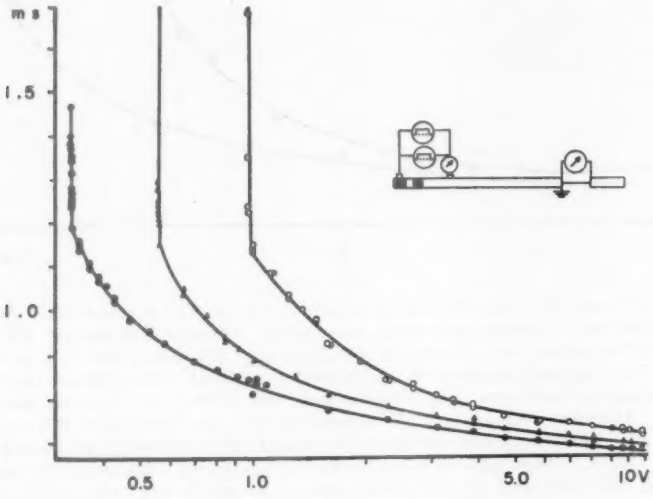

FIG. 5 — *Strength-latency curves during anodal and cathodal polarization (circles and dots, respectively). The intensity of this polarization was 3 μ amp. in both cases. The curve drawn through the triangles shows the control observations without polarization. The duration of the test pulse was 0.4 ms in all cases. Conduction distance: 31 mm. Abscissae: intensity of the test stimuli in volts (logarithmic scale). Ordinates: latency in milliseconds.*

The data from which figure 5 was constructed are replotted in figure 6, but with threshold units as abscissae. Plotted in this form, the three curves practically coincide —i. e., for any intensity of stimulus in terms of threshold, the latency is the same irrespectively of the existence and sign of the polarizing current.

D) *The strength-latency relation during refractoriness.* — In these experiments a cathodal pulse of constant intensity was applied to the nerve with a frequency of 12.5 per second; then, at a certain interval after the first, a second cathodal stimulus of varying intensity was delivered to the nerve and the interval was noted which elapsed between the application of the second (test) stimulus and the appearance of the second response at the recording site. These data allow the construction of voltage-latency and voltage over threshold (V/T)-latency curves at different moments in the refractory period.

The V/T-latency curves obtained at six different moments in the

recovery cycle of a fiber are shown in figure 7. The conduction distance was 31 millimeters. For any intensity of stimulus, the latency increases as the stimuli are delivered earlier during refractoriness. In addition, the curves become less steep as the interval between the two stimuli in each pair (S_1S_2) is decreased and the maximal increment of latency is reduced in magnitude.

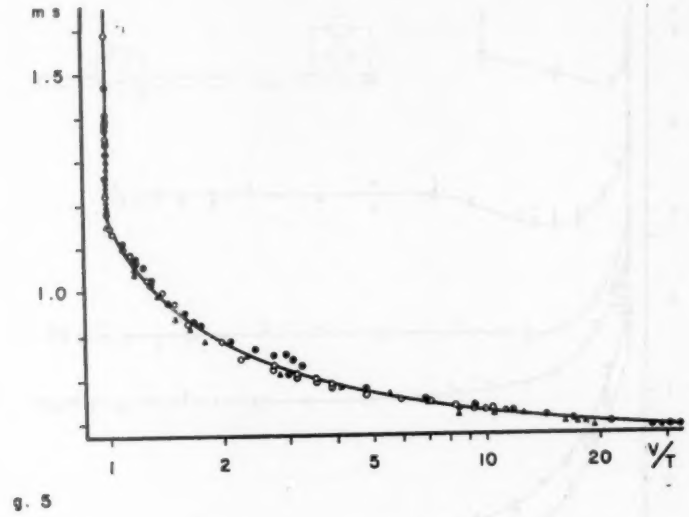

FIG. 6. — *The data in figure 5; but abscissae: intensity of the test stimuli in threshold units (logarithmic scale).*

The curves taken early in the refractory period are quite different in configuration from the normal curve or those obtained later in the recovery cycle. For instance, in the curve taken with an S_1S_2 interval of 0.77 ms (circles in fig. 7) the latency remained practically constant as the intensity of the stimuli was lowered from 21 to about 3 times threshold; a further decrease in the intensity, instead of causing an increase in the latency produced a reduction that persisted down to 1.5 times threshold. In the uppermost curve in figure 7, which was taken with the test stimulus applied 0.60 ms after the conditioning stimulus, a similar phenomenon is faintly perceptible, with the additional feature that in this case stimulus intensities greater than that marked by the arrow did not evoke a second response. Another common finding was that the magnitude of the threshold play of latency decreased as the stimuli were delivered earlier in the refractory period.

In order to gain a better insight into the effects of refractoriness upon the latency of initiation of the action potential, some experiments were performed in which the records were taken at the site of stimula-

tion, thus avoiding complicating factors introduced by changes in the conduction velocity of the impulses. In the first experiments the recording of impulses from the site of initiation was simply accomplished by having a common cathode and ground recording electrode placed at the point of emergence of the single fiber from the nerve trunk. In later experiments the method followed was that described by Katz ([47]) and by Rosenblueth

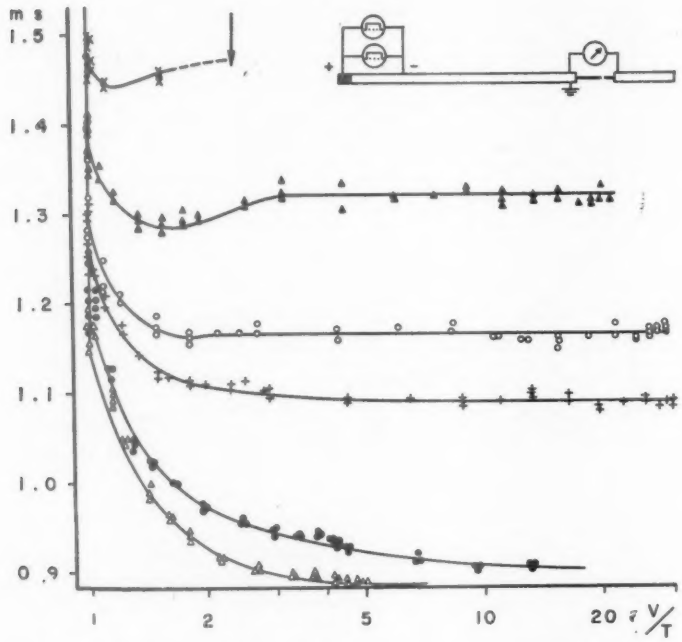

FIG. 7. — *Strength-latency relation at different moments in the refractory period. Pairs of stimuli, conditioning and test. The conditioning stimuli were of 0.1 ms duration and had an intensity of 3.14 times threshold. The duration of the test stimuli was 0.5 ms. Frequency of the pairs, 12.5/s. Conduction distance: 30 mm.*

The interval, in ms, between conditioning and test stimuli was, from the lower to the upper curve: 1.95, 1.40, 0.95, 0.88, 0.77 and 0.60. Abscissae: strength of the test stimuli in threshold units (logarithmic scale). Ordinates: latency in milliseconds.

In the case of the uppermost curve, stimulus intensities greater than that signaled by the arrow did not elicit propagated responses.

and García Ramos ([32]), which gives less shock artifact. For this purpose, the stimulating electrode was placed at the point of emergence of the single fiber and the grounded recording electrode was carried on a microscope stage micrometer and moved along the interpolar stretch of

nerve. Subthreshold pulses of 20 ms duration were applied and a minimization of the passive electrotonic phenomenon was obtained. This was accomplished when the grounded electrode was at a point less than 1 mm from the stimulating electrode placed at the point of emergence of the fiber (see diagram in fig. 8).

The voltage-latency curves obtained from the site of initiation, at

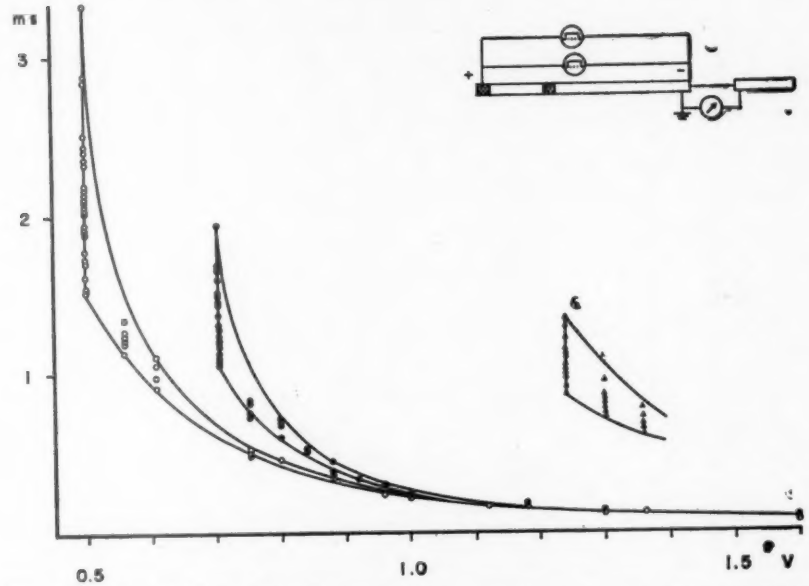

FIG. 8. — *Strength-latency relation at various moments in the refractory period as observed at the site of initiation. Frequency of the pairs of stimuli: 12.5/s. The distance between the stimulating electrodes was 15 mm. The nerve was crushed 4 mm away from the anode (see diagram).*

The duration and intensity of the conditioning stimuli were 0.08 ms and 3.46 threshold. Duration of the test stimuli: 10 ms. The intervals between conditioning and test stimuli were: 70 ms (circles); 2.78 ms (dots) and 1.33 ms (triangles). Abscissae: strength of the test stimuli in volts. Ordinates: latency in milliseconds.

three different moments in the recovery cycle, with the second method described above, are shown in figure 8. In the case of the curve drawn through the circles the interval between the conditioning and test stimuli was about 70 ms while in the one drawn through the full circles it was 2.78 ms; the triangles shown the latency obtained when the interval was 1.33 ms.

The latency of initiation for strong stimuli can not be studied on account of the distortion of the action potential produced by the stimulus artifact. As was expected, it can be seen from the figure that the threshold play of latency is reached with higher voltages as the test

stimulus is moved nearer to the conditioning one. The range of variation of latency with threshold stimulation decreases as the stimulus is applied earlier in the refractory period, and both the maximum and minimum latencies observed with threshold stimuli are reduced. This, and the fact that with high intensities the latency of initiation tends to be almost zero even during refractoriness, decreases the maximal increment of latency as the S_1S_2 interval is reduced.

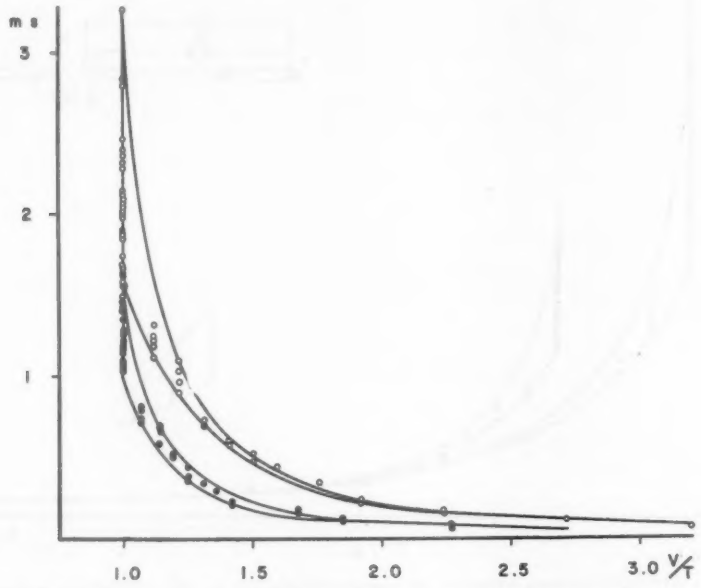

FIG. 9. — *The data shown in the circles and dots in figure 8; but abscissae: intensity of the test stimuli, in threshold units.*

It is also clear from figure 8 that for any selected voltage of stimulus the initiation latency is increased during refractoriness, but this relation does not hold and is inverted if we select an intensity in terms of threshold. Figure 9, which shows the V/T-latency relation for two of the curves illustrated in the preceding figure, shows that the latency for any intensity of stimulus in threshold units is smaller when the test stimulus was delivered 2.78 ms after the conditioning stimulus than when it was delivered 70 ms after. Figure 9 again shows that when the stimulus is many times threshold the latency tends to be the same, independently of whether the stimuli are applied during or after the refractory period.

E) *The influence of temperature.* — In these experiments the nerve fiber was placed in a lucite box filled with mineral oil, and this box was surrounded by iced or hot water. The temperature was read from a thermometer placed in the mineral oil alongside the nerve, and the range studied was from 37 to 8.5° C.

Figure 10 shows the voltage-latency curves obtained at two different temperatures in a fiber in which the conduction distance was 30 mm. Both the threshold and the latency for any intensity of the stimuli are increased by the lowering of temperature while the configuration of the curve is not appreciably changed, although the magnitudes of the threshold fluctuation and of the total variation of latency are increased at the lower temperature.

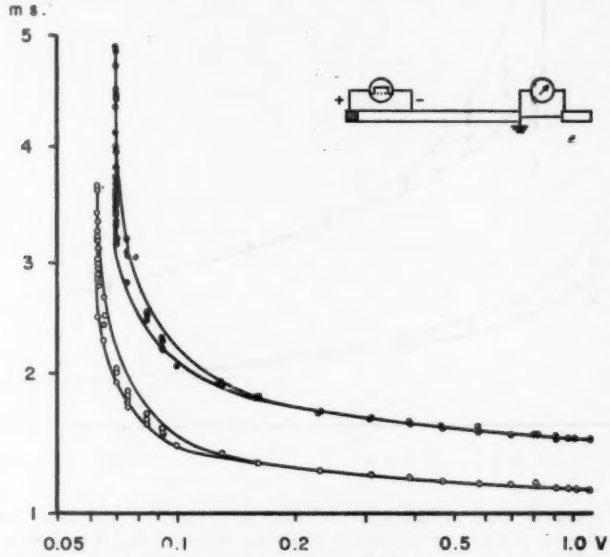

FIG. 10. — *Voltage-latency relations at 17.5° and 10°C (circles and dots, respectively). Same fiber as in figures 1, 2 and 3. Duration of the stimuli: 4 ms. Abscissae: strength of the stimuli in volts (ligarithmic scale). Ordinates: latency in milliseconds.*

The effect of temperature upon the threshold play of latency and upon the maximal increment of latency can also be seen in figure 11, which was constructed from data obtained on another fiber in which the records were taken at the site of initiation, with the balance method described in the previous section. Within the range of voltages studied, for any intensity of stimulus used the latency of initiation of the impulse increased when the temperature was lowered. The same is true for any selected intensity of stimulus in terms of threshold (fig. 12). The difference between the two curves in figures 11 and 12 diminishes as the intensity is increased. It is possible that with very high intensities this difference may disappear; unfortunately, the latency for very strong stimulation could not be studied while recording from the site of initiation.

The effect of changes in the duration of the stimulus upon the strength-latency relation described in section B (figs. 2 and 3) were also found to occur at a temperature of 10°.

F) *The local response and the initiation of the spike potential.* — During a short stay in the Marine Biological Laboratories at Woods Hole,

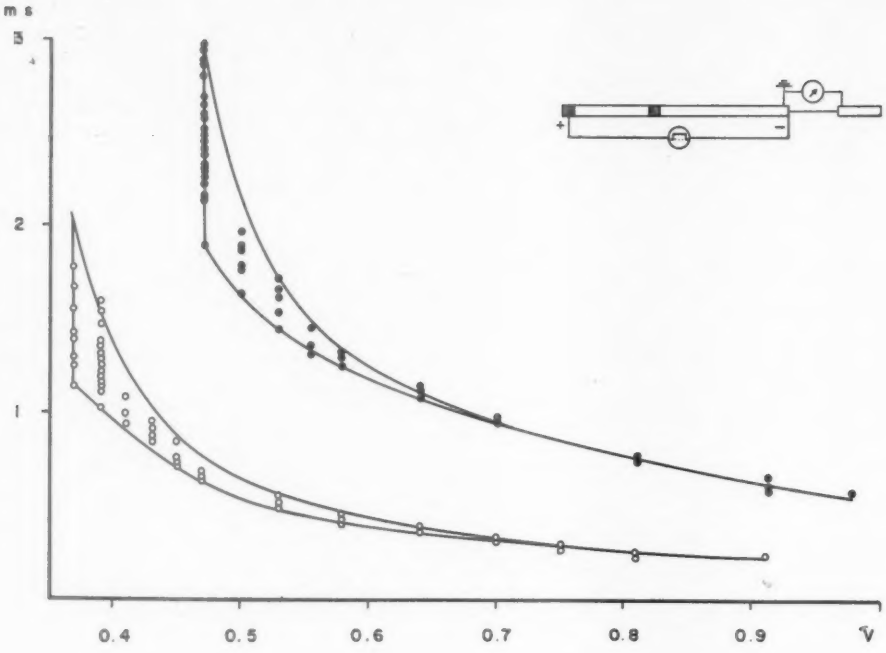

FIG. 11. — *Voltage-latency relations at 22° and 13°C (circles and dots, respectively), as observed at the site of initiation. Distance between the stimulating electrodes: 15 mm. The nerve was crushed 3 mm away from the anode. The stimuli were 10 ms in duration. Abscissae: intensity of the stimuli in volts. Ordinates: latency in milliseconds.*

Mass., in the summer of 1955, we made a few experiments on nonmyelinated axons. We found that the strength-latency relation of axons such as the squid giant fiber and those from the muscle receptor organs of the lobster, is very similar to that of frog myelinated fibers. The local responses of these fibers to stimuli of different intensities and durations were recorded, in order to determine whether the changes in latency were accompanied by concomitant changes in the local responses.

Figure 13 shows the records obtained in a squid giant axon which was carefully freed of all the small fibers and connective tissue that sourrounded it and placed on platinum wire electrodes immersed in mineral oil. The diagram in figure 14 illustrates the method of recording; the distance between the cathode and the proximal recording electrode was of approximately 1.5 mm. The solid line tracings of figure 13 illustrate some of the responses of the fiber to pulses of 10 ms duration, while three of the responses to a just threshold pulse of 10 microseconds duration are shown in broken lines. As the intensity of the stimulus is increased the local response increases in amplitude and its rising phase

becomes steeper. There seems to be no measurable latency for the initiation of the local response. The local responses elicited by stimuli of low intensity are quite constant in form and amplitude, but as the intensity approaches threshold the responses vary greatly in both amplitude and duration. The time that elapses between the application of the stimuli and the moment at which the local responses attain their maximal amplitude increases as the intensity is raised.

Another striking feature shown in figure 13 is the fact that with barely threshold stimuli, when the local response is variable, the derivative of the rising phase of the response shows a decline after which it becomes steeper and the falling phase becomes rapid. The same phenomenon was also seen in the frog fibers and was more conspicuous when the responses were recorded a considerable time after isolating the fiber (one or two days).

The local responses produced by 10 μs pulses are considerably shorter in duration than those obtained with longer pulses. It seems as if with the brief pulses the local response reaches a high level in a short time and then proceeds with a course similar to that of the responses produced

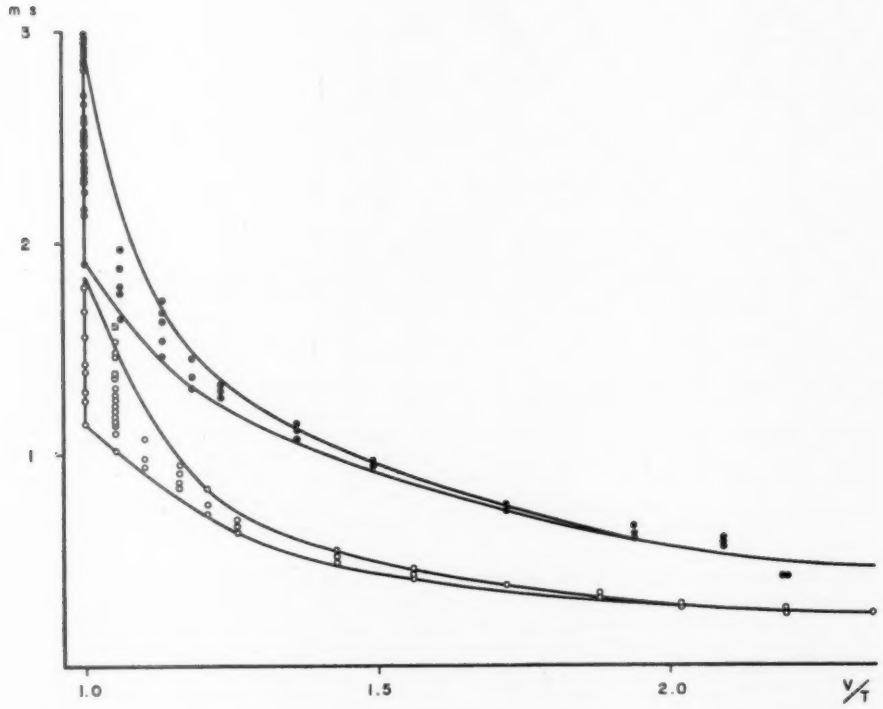

FIG. 12. — *The circles and dots of figure 11; but abscissae: strength of the stimuli in threshold units.*

by stimuli of longer duration. The rising and falling phases of local responses of similar amplitude evoked by stimuli of the two different durations used are practically identical except for their initial portion (fig. 13). The greatest amplitude of local response without spike observed with the two different durations in the experiment shown in figure 13 was practically the same for both.

Figure 14 illustrates in a graphical form some of the results described above. The voltage-amplitude curves of the local response for

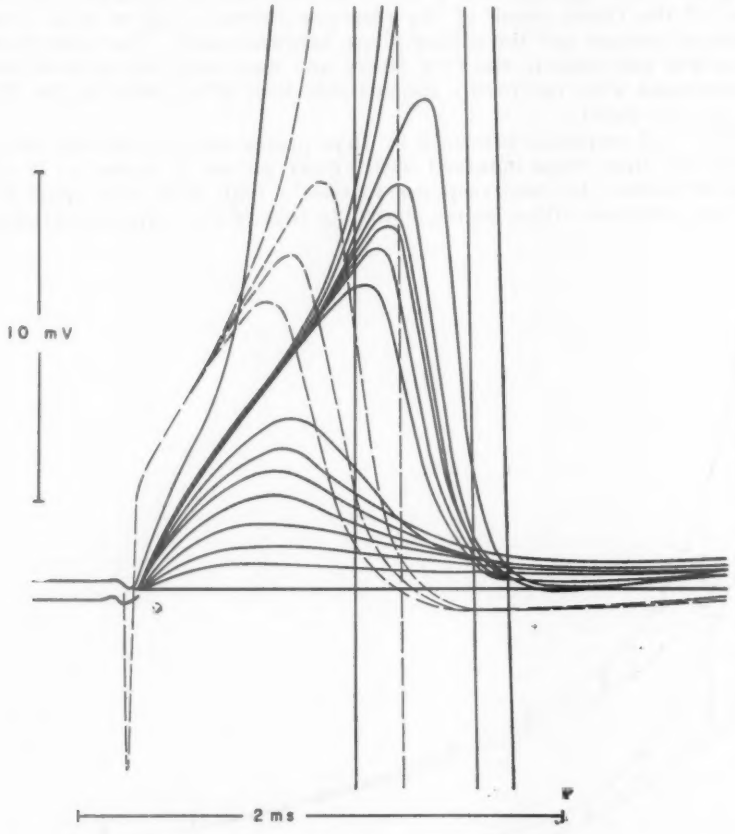

FIG. 13. — *Responses of a squid giant axon to a rectangular pulse of 10 ms (solid lines), recorded as shown in the diagram of figure 14. The 8 lowermost solid-line tracings correspond to the following intensities (in millivolts): 0.01, 15, 25, 35, 45, 55, 60 and 65. The 8 solid-line responses above these were all obtained with an intensity of 70 mv; 2 of them show spikes, as denoted by the fast downward excursions. The additional solid-line tracing with a spike was the response to 150 mv.*

The 4 broken-line tracings are the responses to a briefer pulse (10 μs) with an intensity of 720 mv. Only one includes a spike potential.

the pulses used are similar in form. The only important difference between them is in the scales of the abscissae; an intensity of about 10-11 times greater was needed to obtain with the short pulse the same amplitude of local response as was obtained with the longer pulse. The times from the beginning of the stimulus to the maximum of the local responses evoked by the 10 ms pulses are shown in the curve drawn through the dots; it can be seen that up to 60 mv there was no appre-

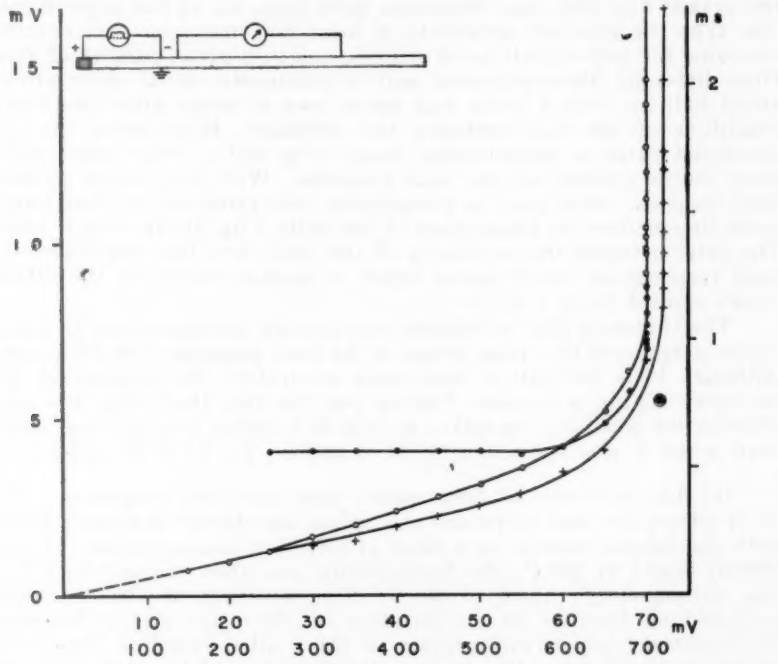

FIG. 14. — *Maximal amplitude of the squid-axon local responses as a function of the intensity of stimulating pulses of 10 ms (circles) and 10 μs (crosses). Ordinates (left-hand scale): amplitude in mv. Abscissae: intensity of the stimuli, in mv; the upper and lower scales correspond to the long and brief pulses, respectively.*

Dots: duration of the rising phase of the responses to the long pulses (right-hand scale of the ordinates), in ms.

ciable change and thereafter the rising time of the local response was prolonged as the intensity was increased. A similar curve was obtained with the short pulse, but the curve was displaced downwards.

The amplitude of the local responses produced at the make of cathodal or at the break of anodal stimuli of different intensities varies in a similar manner in the frog and the invertebrate axons. A considerable variation in amplitude is also apparent in the former as the intensity approaches the threshold value (fig 15). These changes are attended by changes in the

rising time of the response (fig. 16). The steepnes of the rising phase increases if the intensity of the stimulus is increased.

Figure 16 shows the local responses and spikes, as recorded with the balance method, produced by just-threshold stimuli of 20 ms duration (records A-D) and of 0.05 ms (records E-G), obtained in the same frog fiber while it was being stimulated at a frequency of 12.5 per second. Although the intensity of the stimulus for each duration was kept constant, spikes appeared with different latencies and on occasions they were not evoked and only local responses were seen. In all the experiments of this type the greatest amplitude of local response seen was practically the same for both durations of stimuli, and in a great number of records from different fibers examined and on continuous visual observation for about half an hour a spike was never seen to occur after the maximal amplitude of the local response had subsided. Even when the spikes developed after a considerable delay (fig. 16 C), they were seen to start at the summit of the local response. With very short pulses the local response often rose to a maximum and remained at that level for some time before the appearance of the spike (fig. 16, records G and H). The ratio between the amplitude of the spike and the amplitude of the local response at which spikes began to appear varied in the different fibers studied from 7 to 11.

The threshold play of latency was usually accompannied by changes in the steepnes of the rising phase of the local response (fig. 16 A and B). Although it is difficult to determine accurately the moment at which the spike begins, a common finding was the fact that when the latency of initiation was long the spikes started at a higher level of local response than when it was short (fig. 16 A, B and C; fig. 17 B, D and F).

G) *The influence of temperature upon the local responses.* — Figure 17 shows the local responses and spikes for threshold stimuli recorded with the balance method in a fiber at different temperatures. The experiment began at 20° C; the temperature was then lowered to 8.7° and was subsequently raised to 30° C. The lowering of the temperature produced an increase in the duration of the spike and a decrease in its amplitude, which was greater in other fibers studied than in that shown in the figure. The Q_{10} for the duration of the spike, measured at the level of half the maximal height, varied from fiber to fiber. Between 10° and 20° C it fluctuated between 2.2 and 3.0. If the temperature was raised over 30°, and in some cases even at this temperature, the voltage needed to elicit a spike was increased and the amplitude of the spike was reduced.

A decrease in the temperature produced a decrease in the amplitude of the local responses evoked by stimuli of constant voltage; i. e., a greater voltage was needed at low than at high temperatures to obtain the same amplitude of local response. The amplitude of local response at which the spikes were initiated became slightly higher as the temperature was lowered. The total duration and the rising time of the local response were also increased by the drops of temperature (fig. 17).

The time that elapses between the application of the stimulus and the moment at which the local response reaches its maximal amplitude was measured from the largest local responses observed with threshold stimuli at different temperatures. It was found to have a Q_{10} which in

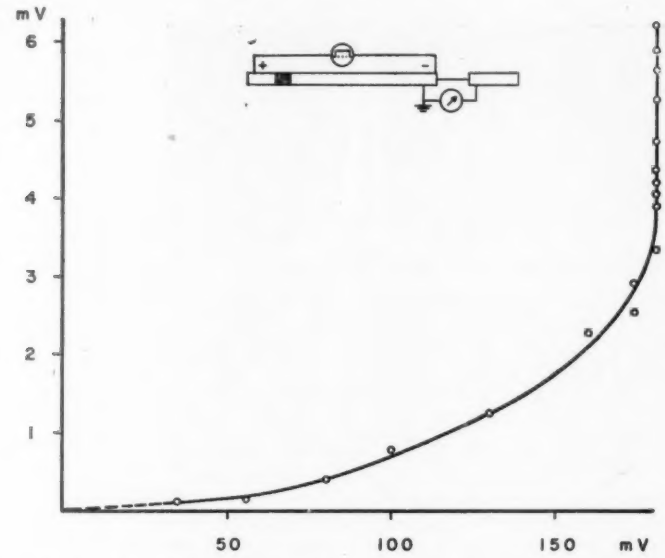

FIG. 15. — *Maximal amplitude of the local responses as a function of the intensity of a 6 ms pulse. Abscissae: intens.ty in mv. Ordinates: amplitude, in mv. The amplitude of the spike was 50 mv.*

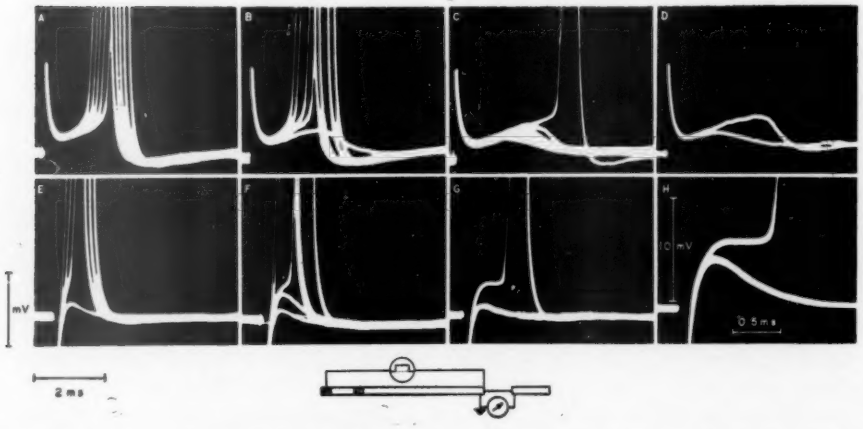

FIG. 16. — *Local responses and spikes elicited by stimuli of different intensities and durations. A to D: duration, 20 ms and intens ty 0.12 v. E to G: duration 0.05 ms and intensity 0.83 v. Record H was obtained from another fiber with a pulse of 0.05 ms duration and intensity 0.86 v. Records taken as shown in the diagram. The calibration marks at the left correspond to records A to G.*

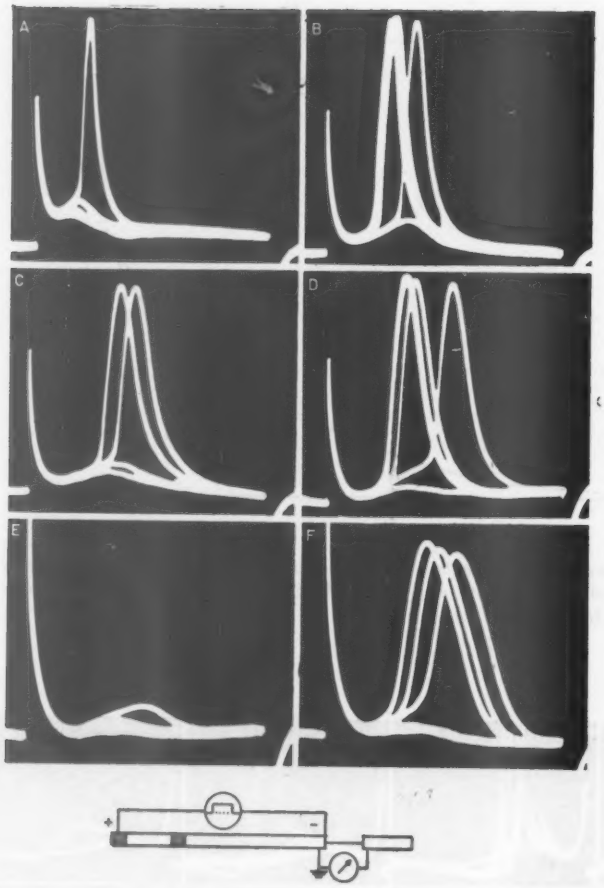

FIG. 17. — *Influence of temperature upon the local responses and spikes evoked by just threshold pulses of 10 ms duration. Same fiber as in figure 8.*

The temperatures to which the fiber was subjected and the intensities of the stimuli were: A, 30° C and 0.442 v; B, 20° C and 0.442 v; C and D, 15° C and 0.45 v; E and F, 8.7° C and 0.56 v.

the fibers studied varied from 1.5 to 1.7. This measurement implies that the local response has no latent period for its initiation (see figs. 13 and 16 A).

An interesting feature shown in figure 17 is the fact that the amplitude of the spike increases as the latency of initiation decreases. The magnitude of this increase in spike height varied in different fibers and was more easly seen at low than at high temperatures.

H) *Repetitive responses and their strength-latency relation.* — In some fibers the application of pulses of long duration and high intensity evoked several responses, while in others the intensity could be raised up to 100 times threshold without evoking more than a single spike. The maximum number of repetitive responses that could be obtained varied from fiber to fiber and in the same fiber in different conditions. In the

cases examined it was never possible to obtain more than five spikes, even though constant current was applied for long periods through AgCl-Ringer-agar electrodes to minimize polarization.

Figure 18 shows typical results obtained in a fiber which gave up to five responses to the application of a square pulse of 20 ms duration. The records include two or three consecutive sweeps and correspond to increasing stimulus intensity. When this intensity was 0.056 v (A) no

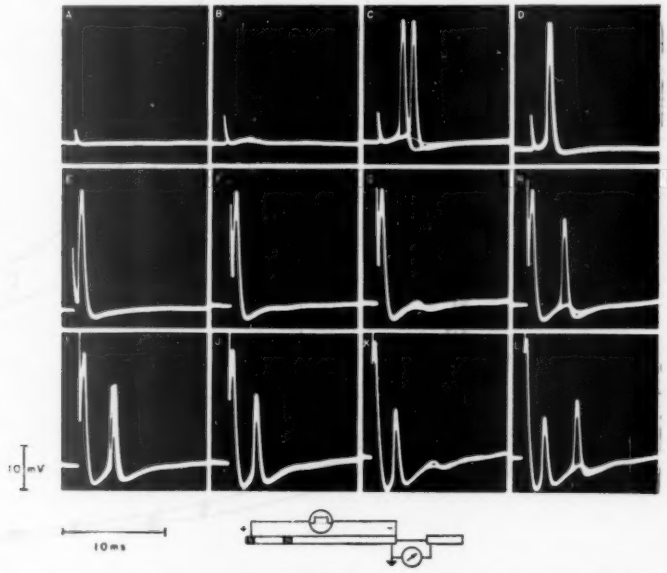

FIG. 18. — *Responses of a nerve fiber to a pulse of 20 ms duration, and the following intensities (from A to L): 0.056, 0.1, 0.112, 0.136, 0.23, 0.39, 0.43, 0.45, 0.47, 0.58, 0.75 and 0.81 v.*

response could be noticed; only the small fast component corresponding to the on of the pulses was seen, the off being out of the oscilloscope screen. With the amplification used a small wave corresponding to a local response was first seen when the intensity was 0.09 v; record B shows this response when the intensity was 0.1 volts. If the intensity was slightly raised the local response varied greatly in form and amplitude and on occasions spikes were evoked with different initiation latencies (C). A further increase of intensity made all the stimuli effective in eliciting spikes and reduced the latency of initiation (D-L). In record F, a small wave followed the positive after-potential of the spike. This second local response later increased in amplitude and duration and its peak occurred earlier. With an intensity of 0.43 v (G) a considerable variation in the form and amplitude of this oscillation was seen, and at a slightly higher voltage (H) a second spike of smaller amplitude was initiated. Like the first spike, this second response appeared after variable delays; it was never seen to occur beyond the peak of the second

oscillation. Further increases in the intensity of the stimulus produced a decrease in the latency of both the first and second spikes and then a third local response (J and K) and spike (L) were elicited and the cycle was repeated until five spikes were included.

The sequence of events described above also occurred at the break of an anodal shock. With cathodal stimuli, in all the cases examined

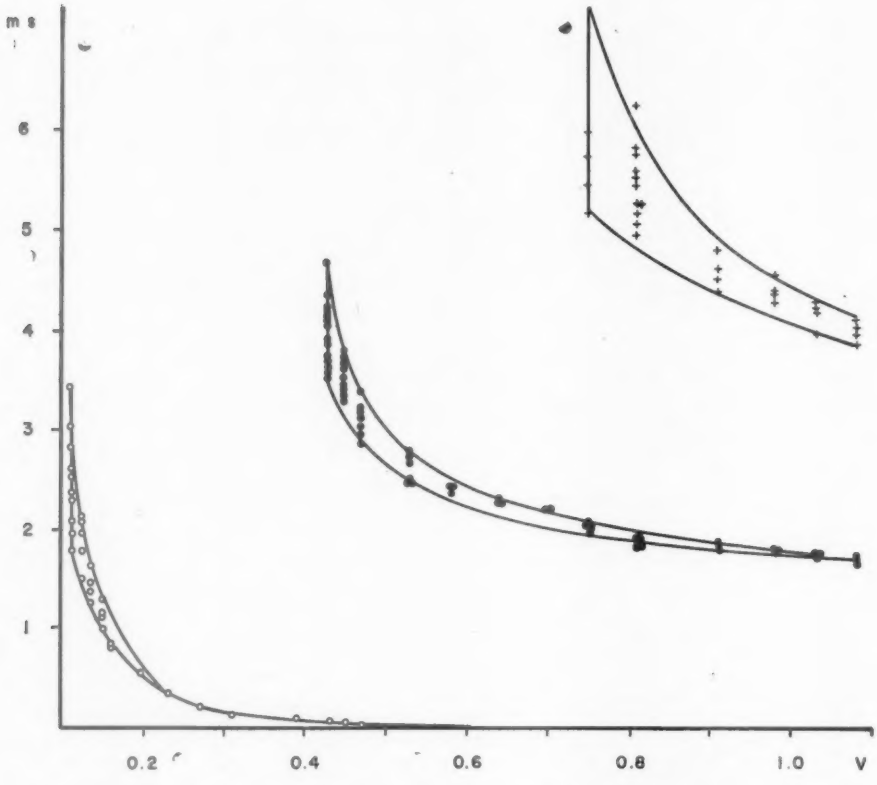

FIG. 19. — *Voltage-latency relations for the three first repetitive spikes (see text) evoked by a stimulus of 20 ms duration. The curves were constructed with the data partly shown in figure 18. Abscissae: strength of the stimuli in volts. Ordinates: latency in milliseconds.*

if the intensity of the pulse was raised beyond that which gave the maximal number of repetitive responses, the spikes were first considerably reduced in amplitude and later some of them could dissappear. With still higher intensities all but the first and occasionally the second spikes were abolished. At this stage the record consisted of the first and sometimes the second spikes followed by a series of damped oscillating local responses.

The results of the experiment partly shown in figure 18 are illustrated in a graphical form in figure 19. From 0 to 0.11 v no spike was elicited. At 0.112 v a spike first appeared and the threshold play of initiation latency was ostensible. As the intensity increased, the latency and its variation decreased, and at 0.43 v a second spike was evoked with variable delays. The latency of the first and second spikes decreased if the intensity was further augmented and at 0.75 v a third spike appeared and the sequence was repeated. In this and all of the experiments on fibers which gave repetitive responses, it was found that the time interval between the first and second spikes was always shorter than that between second and third, and this in turn was smaller than that between third and fourth, and so on.

Discussion

I) *The initiation of spike potentials by electrical stimuli.* — The existence of a local graded response to electrical stimuli was first, indirectly, shown to occur in frog nerve fibers by Rushton [32] and by Katz [37] and later conclusively demonstrated by Hodgkin [38] in single crustacean nerve fibers. Although the existence of local responses in medullated nerve fibers has been denied [Blair and Erlanger [36], Blair [38]], they have been recorded in frog fibers by Katz [47] and by Schoepfle and Erlanger [50, 51]. A detailed study of the local responses of myelinated nerve fibers of cats under different conditions, has been made by Rosenblueth and Luco [50], Rosenblueth and García Ramos [51, 52] and García Ramos and Calva [52].

The relation between strength of stimulus and maximal height of local response illustrated in figure 14 was first suggested for brief stimuli by Katz [37] and has been confirmed on single unmyelinated nerve fibers [Hodgkin [37, 38]; Ledingham and Scott [38]; Pumphrey, Schmitt and Young [40]], on mammalian myelinated fibers [Rosenblueth and Luco [50]] and on single frog nerve fibers [del Castillo and Stark [52]]. The relation between the strength of rectangular pulses of long duration and the amplitude of the local response, is similar to that prevailing for short pulses (figs. 14 and 15) except for the fact that with the brief pulses a greater intensity is needed to obtain the same amplitude of local response as obtained with pulses of long duration.

Schoepfle and Erlanger [51] state that a local response develops only when the intensity of the stimulus is over 0.85 threshold for spike discharge. In the experiments of Katz [37], Hodgkin [38], Ledingham and Scott [38], Pumphrey, Schmitt and Young [40] and del Castillo and Stark [52] the local response attained a measurable amplitude when the intensity of the stimulus was about 0.5 threshold. On the basis of these results these authors conclude that there is a threshold for the production of a local response. On the other hand Katz [47] observed local responses in the frog's sciatic nerve with stimulus intensities of 0.3 threshold and Luco [50] reports having measured local responses in cats' spinal rootlets with strengths of 0.36 threshold. These observers reached the conclusion that there is no threshold for the local response. The present results show that if this threshold exists it should be less than about 0.25 that for spike discharge (figs. 14 and 15).

The variation in the amplitude and duration of the local responses evoked by stimuli of constant intensity and their increasing fluctuation as the intensity approaches the threshold value is a phenomenon common to both the squid and frog axons (figs. 14, 15 and 16). It had been previously described in single fiber preparations [Hodgkin ([38]), Schoepfle and Erlanger ([51]), del Castillo and Stark ([32])]. The fact that it was not seen in nerves composed of many fibers [Katz ([47]), Rosenblueth and Luco ([30]], may be due to a smoothing out effect from the many fibers responding. It is this fluctuation that causes the occasional failure in the initiation of spikes when a just threshold pulse is used.

That the duration of the local response and its rising time increase as the intensity of the stimulus is increased was suggested in the study of Katz ([37]). These facts were confirmed by Rosenblueth and Luco ([50]). The relation between the duration of the rising phase of the local response, and the strength of the stimuli is shown in figure 14. This phenomenon may be due to the intervention of a greater membrane area as the intensity of stimulation is increased.

As shown in figure 13, the non-propagated responses to nearly threshold stimuli appear as if they were composed of two superimposed waves. Three possible explanations are suggested.

a) This duality might be fictitious and the tracings may correspond to a complex time-course of a single process, namely, a local response different in nature from the spike potential [see Rosenblueth and Luco ([50])].

b) An "abortive spike" might be superimposed on the local response [see Hodgkin and Rushton ([46])].

c) A process, different from those responsible for the development of the local response and the spike potential, and intermediary between them, might be revealed by the additional wave in question.

No conclusive evidence is available to discriminate between these possibilities. The following considerations, however, are apposite.

According to Hodgkin ([38]) the local response and the spike potential are akin; they correspond to different degrees of a single process. Whether or not this be the case, if the responses in figure 13 include spike potentials then these data lead to the conclusion reached by Averbach and Nasonow ([50]) that the spike potential does not obey the all-or-nothing law.

Hodgkin ([51]) points out that the existence of a local response may be readily explained if there exists a graded and reversible relation between membrane potential and sodium conductance, for in this case a small depolarization would produce a slight increase in sodium permeability such that the inward current due to sodium entry is less than the outward current due to the movements of potassium and chloride ions. He defines the membrane threshold as the potential at which the inward current due to sodium exceeds the outward current due to potassium and chloride. Above this critical depolarization the excess inward current would continue to depolarize the membrane and exert a regenerative action on the sodium carrier mechanism. If the change in slope of the rising phase of the local response is due to the production of an "abortive" spike, additional postulates must be made to the theory to explain why

that spike does not attain its full size, since the depolarization has already crossed the critical level.

Hodgkin ([38]) suggested that the threshold for the initiation of spikes is reached when the local response attains a critical amplitude; a suggestion that has been widely accepted. A different hypothesis has been proposed by Rosenblueth ([52]), who thinks that the decisive factor for the initiation of a spike is the attainment of a critical quantity, as opposed to a critical amplitude. This hypothesis is based on the observations of Rosenblueth and Luco ([50]) and of Rosenblueth and García Ramos ([51], [52]) which show: a) that the amplitude of the just-threshold local responses to anodal or cathodal pulses of different durations varies importantly; b) that with low intensity of stimulation the spikes start after the local response has subsided significantly; and c) that the amplitude of the threshold local responses to alternating currents of different frequencies is not constant while the areas are constant for frequencies ranging from 50 to 600 cycles per sec. The present results show that in the squid and frog axons the amplitude of the threshold local responses for rectangular pulses of widely different durations is practically the same (figs. 13 and 16) and that the impulses evoked by stimuli of different durations, applied to fibers in different conditions of refractoriness and temperature, never started when the peak amplitude of the local responses had subsided. If quantity of local response is the important parameter in the production of spikes, they should have always appeared, with threshold stimuli, at the completion of the local response; i. e., on the terminal portion of the descending phase. Furthermore, with threshold stimulation, when the latency of initiation is varying importantly the total area of local response underlying a spike is many times greater when the spike is considerably delayed than when it appears early after the application of the stimulus.

The divergence between these results and those of Rosenblueth and collaborators may be attributed to the fact that they worked on nerve strands composed of many fibers, for in this case the local response recorded corresponds to the sum of the individual responses. Since the fibers have different excitabilities, and as has been mentioned above, the amplitude, rising time and total duration of the local response increases with the intensity of the stimulus, it is expected that the maximum of the compound local potential will not occur at the same time as that of the most excitable fiber but this, instead, should occur later, and so when the critical amplitude of local response for this fiber is reached, a spike will appear on the descending phase of the compound local response. It can be concluded that a necessary condition for the initiation of a spike potential is the attainment of a critical amplitude of local response.

When just threshold cathodal rectangular pulses of long duration are applied to a nerve fiber, the critical amplitude of local response at which the spike potentials are generated is greater, the greater the latency of initiation (see section F). A similar phenomenon appears in single muscle fibers from the frog's sartorius [Jenerick ([36])]. We may consider this rise in the critical amplitude of local response during the application of a long pulse, as one of the manifestations of accommodation; the other is the decrease, in the course of the application of the

long pulse, of the amplitude of the local response evoked by a stimulus of constant intensity [Rosenblueth and García Ramos ([52])].

Another phenomenon observed during the threshold play of latencies was the increase in the amplitude of the spikes when they were initiated early after the application of the stimulus; if the intensity of the stimulus was raised over threshold the spike amplitude was further increased. This seems to be a phenomenon of general occurrence among excitable tissues, since it has been shown to occur in striated muscle fibers [Jenerick ([56])] and it can also be seen on examination of some of the records obtained in the squid axon by Hodgkin, Huxley and Katz ([52]) (figs. 8 and 9) and in those of Altamirano et al. ([94]) (fig. 13) obtained from the denervated electroplaque.

Both the increase in the amplitude of the critical local response and the decrease in the amplitude of the spike that are observed as time progresses after the application of a rectangular pulse of long duration may find an explanation in the "inactivation" and increased potassium conductance which occur after a steady depolarization in the squid axon [Hodgkin and Huxley ([72] a and b)]. These two factors would tend to reduce the amplitude of the spikes initiated late after the onset of the stimulus. The increase in the amplitude of the local response necessary to initiate a spike can be attributed to the greater depolarization needed to exceed the increased potassium current in order to generate a spike.

A different explanation of the reduced amplitude of the late spikes arises, if as maintained by Rosenblueth and collaborators [see Rosenblueth ([92])], different processes underlie the production of local responses and spikes. In this case if we assume that the local response does not disappear after the production of a spike potential, or that it does so only after the spike has reached its maximum, then it may be that the early spikes appear greater because they are superimposed on ampler local responses (as suggested by their increased ascending slopes). Furthermore the peak of the spikes initiated after a considerable latency may fall on the descending phase of the underlying local response and so their sum would be smaller. More data are needed in order to discriminate between this explanation and the one offered in the preceding paragraph.

II) *The strength-latency curve.* — Since a spike potential is generated as soon as the local response exceeds a critical amplitude, it is clear that the latency of initiation of impulses will depend on the mutual relations existing between the critical level and the time development of the local response. With this in mind we can proceed to explain the variations in latency that accompany changes in the intensity of the stimulus.

If the intensity of the stimulus is low, only a small local response, that does not reach the critical amplitude, is produced. If the intensity is increased, the amplitude, rising time, total duration and slope of the rising phase of the local response are increased. As the intensity is approached, at which the amplitude of the local response and the critical level are of the same magnitude, an increasing fluctuation of the form and amplitude of the local response is seen. When this intensity, that we call threshold, is reached, the local response of varying amplitude will sometimes exceed the critical level and generate a spike and at

others it will fail to do so because it does not attain the critical amplitude. Since the variation in the amplitude of the local response is a random variation, it is clear that the spikes will be evoked at random (see section A). This will happen even if the critical level does not show any fluctuations, a matter that has not been studied here; in any case, if this fluctuations occur they are small compared to the variations in amplitude of the local response.

The threshold play of latency can be readily explained if we take into account the variations in the steepnes of the rising phase of the local responses evoked by threshold stimuli (section F), for it is clear that the steeper local responses will reach the critical level sooner and produce spikes with smaller latencies than the slowly rising local responses. The random threshold latency variation (section A), finds an explanation in the random variation of the slope of the local response.

If the intensity of the stimulus is progressively increased the ascending slope of the local response will be correspondingly increased, and so it will cross the critical level at shorter intervals after the beginning of the stimulus and the latency of the impulses will be reduced. If we assume that the steepnes of the local response has an upper limit or that it approaches verticality up to the critical level, then a moment will be reached, when any further increase in the intensity of the stimulus will not produce any change in the latency (fig. 1).

Tasaki and Fujita ([48]) and Tasaki ([53]) indicate that the strength-latency relation for long pulses fits with fair accuracy the hyperbola $v/b = 1 + (k/t)$, where v represents the voltage applied, t the latency of initiation, and k and b are constants; the latter being the rheobase. The strength-latency curves obtained in the present experiments are approximately represented by that relation in the segment from threshold up to about 3 times threshold; with greater intensities the curve may deviate importantly from this relation.

III) *The influence of the duration of the stimulus upon the latency of initiation.* — Since the amplitude of local response necessary to evoke a spike is practically the same for stimuli of different durations and since the rising time of local responses of the same amplitude elicited by short pulses is briefer than that for long pulses, then with brief stimuli the threshold local response will reach the critical level sooner and will produce a spike earlier than with pulses of greater duration (figs. 2 and 3). The fact that within a certain range of intensities the latency for a brief pulse is greater than that prevailing for a longer pulse (fig. 2), may be explained by the fact that for the same intensity of stimulus the local response evoked by the brief pulse is smaller and is less steep (except for its initial portion) than the local response evoked by the longer pulse. For very high intensities of stimulation the steepness of the local responses produced by both the short and long pulses tends to reach the same limit; the impulses initiated by them, therefore, will have approximately the same latency (fig. 2).

The relation between amplitude of local response and strength of stimulus is similar for pulses of short and long duration (fig. 14). It may be assumed that the relation between strength of stimulus and slope of the local response will also be similar. The local response produced by a very short pulse will rapidly build up to a level that depends on the

intensity of the stimulus, and from there on it will proceed with approximately the same time course of the local response evoked by a long pulse of the same relative intensity (fig. 13) in threshold units and will therefore reach the critical level and generate a spike sooner than the long pulse. This will happen until the limit in the steepnes of the initial portion of the local response is reached; at this point a stimulus many times threshold will evoke a spike with a latency that will be independent of the duration of the stimulus (fig. 3).

The smaller threshold play of latency observed as the duration of the stimulus was decreased (section B, fig. 3) may be explained on a similar basis. We have only to assume that the threshold variation in the slope of the local responses is independent of the duration of the stimulus. If this is so, it is then clear that since the local response produced by brief pulses has a rapid initial portion, then for the same variation in slope, the range of points at which these responses cross the critical level will be smaller than that corresponding to long pulses.

Since the spikes are generated when the local response reaches a critical level and before it decreases in amplitude, the total variation of latency (see section B) is in a way a measure of the rising time of the local response. If the responses are observed at their site of origin then the slowest rising time of the local response is equal to the greatest latency observed. In the present experiments the greatest latency recorded at the site of initiation was of 6.08 ms with a pulse of 20 ms duration in a fiber at 24.2° C.

IV) *The effects of polarizing currents.* — The present observations on the effects of polarizing currents upon the threshold of nerve fibers confirm those reported by Suzuki, Ando and Miyata ([54]). It was found (section C) that after an initial decrease, the threshold during cathodal polarization increased and could even become higher than normal; while after the initial increase observed during anodal polarization, the threshold decreased but did not go below the normal level. The changes in threshold that are seen immediately after the beginning or end of the polarizing currents may be due to the development of local responses or positive swings produced by the on and off of the polarizing current [Rosenblueth and García Ramos ([52])].

The late increase of threshold during cathodal polarization may be due to an increase of the critical level of local response necessary for the initiation of impulses, to a decrease in the amplitude of the local responses, or to a combined action of both factors. That a decrease in the amplitude of the local response plays an important role in the production of this phenomenon is indicated by the results of Rosenblueth and García Ramos ([52]) who found that the local responses to brief shocks of constant intensity gradually diminish in amplitude during the application of a long cathodal pulse and may even become smaller than the controls. The progressive decrease in threshold observed during anodal polarization may in turn be due to a decrease of the critical level and to an increase in the amplitude of the local response [Rosenblueth and García Ramos ([52])]. Since with anodal polarization, the threshold, after its initial rise is only slightly reduced down to a level that depends on the intensity of the polarizing currents, the increase in the latency of the impulses evoked by pulses of constant intensity observed as the polarizing current

was intensified (fig. 4) may be due to a reduction in the relative amplitude of the local response until it reaches threshold value.

The changes in the strength-latency curve produced by polarizing currents (figs. 5 and 6) can not be fully explained until more is known on the behavior of the local responses evoked at different moments after the application of polarizing currents of different intensities.

V) *The latency during refractoriness.* — Some of the changes in the strength-latency relation that are observed during the recovery period (figs. 8 and 9) may be explained by the reduced amplitude of the local responses evoked in the refractory period [Hodgkin (38); Pumphrey, Schmitt and Young (40); Rosenblueth and Luco (50); Rosenblueth and García Ramos (52)]. Since the critical amplitude of local response at which the spikes are generated is increased during refractoriness [Hodgkin (38); Rosenblueth and Luco (50)] we have to assume an increase in the slope of the rising phase of the local response to explain the smaller latency of the spikes generated in the refractory period by stimuli of the same intensity in terms of threshold.

The increased latency observed during refractoriness for any intensity of stimulation when the potentials are recorded at a distance from the stimulating electrodes (fig. 7), is to be explained by the fact that the delay includes then the latency of initiation plus the conduction time, and this is known to increase as the impulses are propagated more and more in the refractory period [Gasser and Erlanger (25)]. If the test stimulus is applied early after the conditioning one, the latency of initiation will increase as the intensity is decreased, but the conduction time will be reduced on account of the greater interval between the impulses; the form of the strength-latency curve will then depend on the relative magnitude of these two factors. If for any decrease in intensity the increase in latency of initiation is smaller than the decrease of the conduction time the relation will have the shape shown in the upper curves of figure 7.

VI) *Temperature and latency.* — The increase in the latency of initiation that is observed as the temperature is lowered (figs. 10 and 12) confirms the findings of Tasaki and Fujita (48), the only difference being that in their experiments the rheobase was not affected by temperature changes. This increase in the latency may be explained by the decrease in the amplitude of the local responses and by their slower rising phase (see section G and fig. 17).

It is possible that with very high intensities of stimulation the latency is independent of the temperature, as suggested by the fact that, at the site of initiation, the latency curves obtained at different temperatures tend to approach each other (figs. 11 and 12). If this were so, then the difference, in latency for strong stimulation shown in figure 10 is to be attributed to the slower conduction velocity that is observed at low temperatures. Unfortunately, in these experiments the conduction velocity was not measured after the temperature was changed and so the conduction time can not be deducted from the total latency measurements.

VII) *Repetitive responses.* It was mentioned in section H that, under the same experimental conditions, some of the frog fibers responded with repetitive activity to the application of pulses of long duration, while in others the intensity of a long pulse could be raised

greatly over threshold without evoking more than a single spike. A detailed account of repetitive activity will be presented in a future communication. We merely mention here that in some fibers a series of damped oscillatory local responses could be seen after the first spike was generated. In other fibers, as the stimulus intensity was increased only one oscillation was produced after the first spike; this oscillation increased with the intensity of the stimulus until it produced a second spike and then a third one appeared. It seems as if the generation of a spike favours the development of a subsequent local response. A similar phenomenon has been previously described in auricular heart muscle by García Ramos and Rosenblueth ([47]).

The repetitive responses were never seen to occur on the descending phase of the corresponding local response. The strength-latency curves of the repetitive spikes are similar to that of the first response.

SUMMARY

The latency of initiation of impulses was studied in frog single myelinated nerve fibers, and a few experiments were made on single unmyelinated fibers from the squid and from the lobster.

The latency decreases as the intensity is increased, and this decrease has a limit which is reached with intensities of from 30-80 times threshold (fig. 1 A). At room temperature the maximal increment of latency for the different fibers studied ranged from 1.0 to 6.0 ms.

With threshold stimuli the latency for consecutive responses varied greatly and at random (section A). The range of this play of latencies was decreased if the duration of the stimulus was decreased (fig. 2), or during refractoriness (figs. 7 and 8); and it was increased at low temperatures (figs. 10 and 11). At room temperature it varied in the different frog fibers studied from 0.55 to 4.05 ms.

For a given voltage, the latency was smaller the longer the duration of the stimuli, while for a given intensity in threshold units the latency was smaller the shorter the duration (figs. 2 and 3). With very high intensities of stimulation the latency reached a limit which was independent of the duration of the stimulus.

During the application of cathodal polarizing currents the threshold, after an early decrease, increases and may even become greater than normal. With anodal currents the threshold first increases and then decreases (section C).

The latency increases with the intensity of anodal polarizing currents (fig. 4). During anodal polarization the latency is increased, as compared to the control, for a given intensity of stimulus (fig. 5). Cathodal polarization may either increase or decrease the latency for a given voltage of stimulation, depending on the intensity of the polarizing current and on the time after the onset of polarization at which the test stimuli are applied (section C). For any selected intensity of stimulation in threshold units, the latency does not change with either anodal or cathodal polarization (fig. 6).

The changes in the strength-latency relation during refractoriness were studied. The threshold play and the total variation of latency were decreased during refractoriness. For a given voltage of the stimuli the

latency is increased during the refractory period (fig. 8). With a fixed intensity in threshold units, however, the latency decreases during refractoriness (fig. 9). With high intensities the latencies at different moments in the recovery cycle tend to reach the same limit.

Lowering the temperature increases the latency of initiation, the threshold play and the maximal increments of latency (figs. 10 and 12). The threshold increased as the temperature was lowered.

In some fibers repetitive firing was observed when a stimulus of long duration and sufficient intensity was applied. The strength-latency curves for these repetitive responses are similar in shape to that of the first response (fig. 19).

The role of the local response in the initiation of spikes, was studied. It is concluded that the attainment of a critical amplitude of local response is a necessary condition for the generation of impulses. An attempt was made to explain the strength-latency relation on the basis of this conclusion.

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ON THE EFFECT OF ADRENALINE ON ISOLATED INTESTINAL SMOOTH MUSCLE

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It has been suggested that the relaxing effect of adrenaline on some types of smooth muscle is an indirect one and due to the action of some metabolic product. Mohme-Lundholm's (1953) observations on isolated coronary vessels, intestinal and tracheal smooth muscle suggested that the relaxing effect of adrenaline is due to the ability of this substance to stimulate lactic acid production since: a) its effect coincided with an increase in the lactic acid content of the tissue, b) this acid has a relaxing action, c) the effects of adrenaline and those of lactic acid were abolished by alkalyzing the bathing solution, and d) there was inhibition of the adrenaline relaxation when lactic acid production was blocked by several types of enzyme inhibitors. Lundholm and Mohme-Lundholm (1957) reported that lactic acid production and the relaxing effect of adrenaline were associated with an increase of glycogenolysis, and that this three phenomena vary in a parallel way in several different conditions.

It seems thus clear that the relaxing effect of adrenaline on smooth muscle is intimately related with the metabolic action of this substance, and therefore is not a result of a direct effect on the contractile mechanism. On the other hand, it is known that both adrenaline and lactic acid, in small doses, may have a stimulating action on the smooth muscles in which they usually produce relaxation (García Ramos, Alanís and Luco, 1950; see also Mohme-Lundholm, 1953).

The present is a study of the factors which may have a more or less independent influence upon either the metabolic or the contractile mechanism, and of how they affect the responses of intestinal smooth

* This study was initiated by a group of students of the "Escuela Médico-Militar de México" as part of their regular training in experimental physiology.

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muscle to adrenaline. There are also some additional data on the effect of lactic acid and sodium bicarbonate.

METHOD

Segments of the upper portion of the small intestine of the rabbit were used throughout. The animals were anesthetized with pentobarbital given intravenously. The anesthesia was used in order to have at hand a new and fresh segment whenever necessary; it was without appreciable effects, since similar results were obtained when intestinal segments were taken from the animal at different levels of anesthesia, or at different moments from the beginning of the experiment, or when the same segment was used for longer periods of time after several washings. This last condition presented an exception that will be described later, but which does not invalidate the assertion that anesthesia do not constitute an important complicating factor.

The isolated segment (15 to 20 mm long) was tied from one end in an ordinary bath, but modified to include a device for simultaneous determination of the rate of oxygen consumption (fig. 1).

On some occasions, the intestinal segment was tested with the mucosal side out. This will be referred to later as the "inverted" preparation.

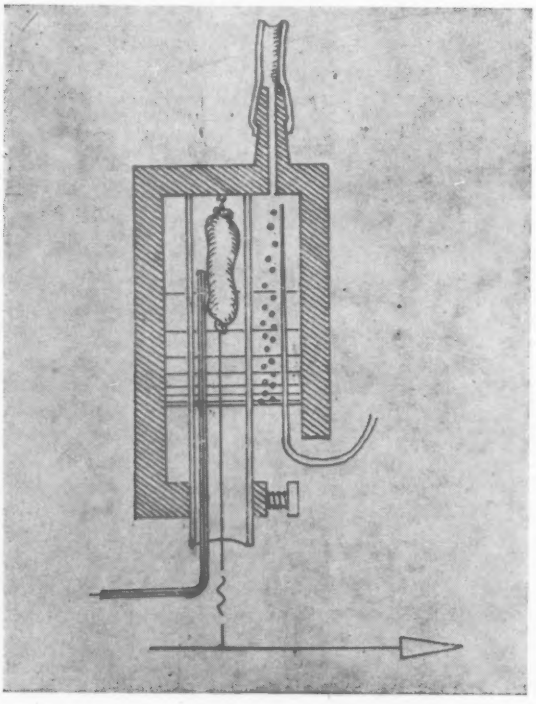

FIG. 1. — *Diagram showing the modified chamber used in measurements of rate of oxygen consumption. The inner glass tube is shown in its down position as in the actual measurement. Only the platinum electrode is shown in place. The calomel cell was connected to the bathing solution by a plastic tubing filled with Ringer's.*

The mechanical activity was recorded on a kymograph with an isotonic lever, with a load of 1 g. The rate of oxygen consumption was determined by the polarographic method. A platinum, glass insulated electrode with the tip covered by a collodion coating, was placed near the intestinal segment. Between this and a calomel cell a polarizing voltage of 0.6 to 0.8 volts was applied. The current in this circuit is directly proportional to the oxygen tension of the solution. The voltage drop produced by that current through a selected resistance was amplified (Grass, direct coupled amplifier), and recorded with a microammeter as a point every two seconds, on paper driven at constant speed. For two minutes, out of every four or five, the chamber enclosing the preparation and the oxygen electrode were separated from the continuosly aereated solution by lowering the inner glass tubing and sealing the resultant closed compartment with mineral oil. As the intestinal segment was using oxygen from this enclosed solution, the oxygen tension fell with a rate proportional to the rate at which oxygen was being consumed by the tissue.

The whole chamber was immersed in a constant temperature water bath, and the solution was constantly bubbled with atmospheric air. The bathing Ringer's solution had the following composition per liter: NaCl, 7.96; KCl, 0.417; $CaCl_2$, 0.271; NaH_2PO_4, 0.046; Na_2HPO_4, 0.186 g, and had a pH of 7.2. The solutions of adrenaline were prepared at a concentration of 10 micrograms per mililiter in distilled water, kept in a brown glass bottle during the day of the experiment, and used in most of the observations in order to have a final concentration of 0.1 of a microgram per mililiter. A fresh solution was used every day, even if in that condition the rate of oxidation is very slow (it looses in ten days less than 50 per cent of its activity, at an average room temperature of 20° to 22° C). Cyanide and azide were used as their sodium salts. The lactic as well as the acetic acid were used dissolved in buffered Ringer solution at a concentration of 10 µg per ml, and the sodium bicarbonate as a saturated solution in Ringer's. From those mother solutions the required amount was added to the solution in the bath.

The rate of oxidation of adrenaline was followed spectrophotometrically, using a light of 510 mµ wave length, on a Beckman DU Spectrophotometer.

The electrical records were taken from an intestinal segment extended over the bottom of a Petri dish, under some tension to avoid movement artifacts. The preparation was surrounded by a small amount of fluid and the dish covered to prevent dessication of the tissue. In most cases the peritoneum was removed at the site in which the recording electrodes were placed. These were fine platinum wires just touching the muscle surface exposed. A capacity coupled preamplifier, with a time constant of 1 sec, was used. The records were photographed from the screen of a Philips cathode ray oscilloscope.

Results

A) *The effects of temperature.* — Temperature affects the rate of oxygen consumption in the same way as described for other tissues. The method used does not permit to present quantitative data.

To describe the effects of temperature on the mechanical activity it is necessary to separate those on the frequency from those on the amplitude of the contractions. For the temperature range studied (16° to 45° C), the frequency of the oscillations increased linearly with temperature. The response to temperature is slow. It takes several minutes after a change in temperature for the preparation to reach an equilibrium with it. As an average for different preparations, the temperature coefficient for the increase in frequency was 2.5; it varied between 2.1 and 2.7.

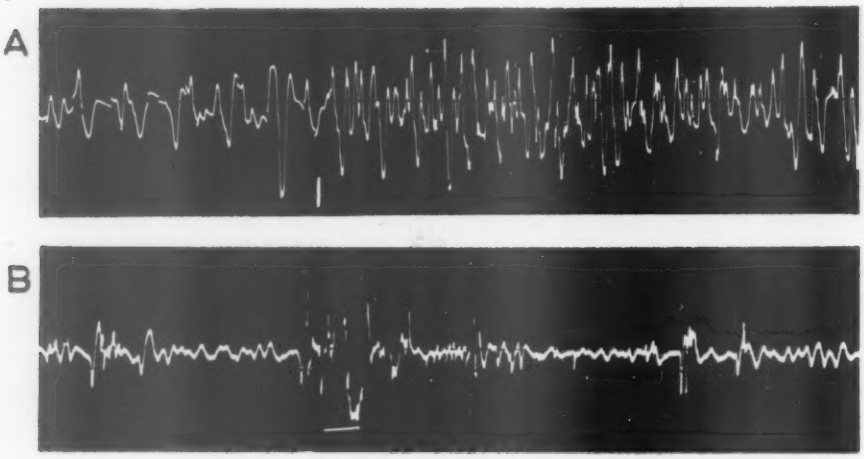

FIG. 2. — A. *Effects of adrenaline (1 microgram per millilitre at the vertical bar) on the intestinal smooth muscle potentials. The concentration of this substance was 10 times greater than that used in most of the observations with mechanical records. This amount will have produced a complete inhibition of the intestinal movements.* B. *Effects of lactic acid (0.2 micrograms per millilitre) on the electrical records. The large oscillations at the horizontal bar were artifacts produced by the adding of the lactic acid solution and the removal of its excess between the recording electrodes. Time calibration 1 minute.*

Quantitative effects of temperature on the amplitude of the oscillations are more difficult to establish. In this kind of preparation, the amplitude of a contraction depends on several factors: a) the number of longitudinal fibers being active at that moment, b) the extent of their degree of shortening. Non-active fibers may damp the movements since they can be passively stretched, c) the degree of automaticity of the preparation. With an increase in temperature new pace-makers or foci of automatic activity may appear, and the lack of synchronization between them will give many irregularities in the frequency and therefore in the amplitude of the contractions. Furthermore, a rise in temperature increases the rate of oxygen consumption and this may create hypoxic conditions for the higher temperatures, particularly when the solution

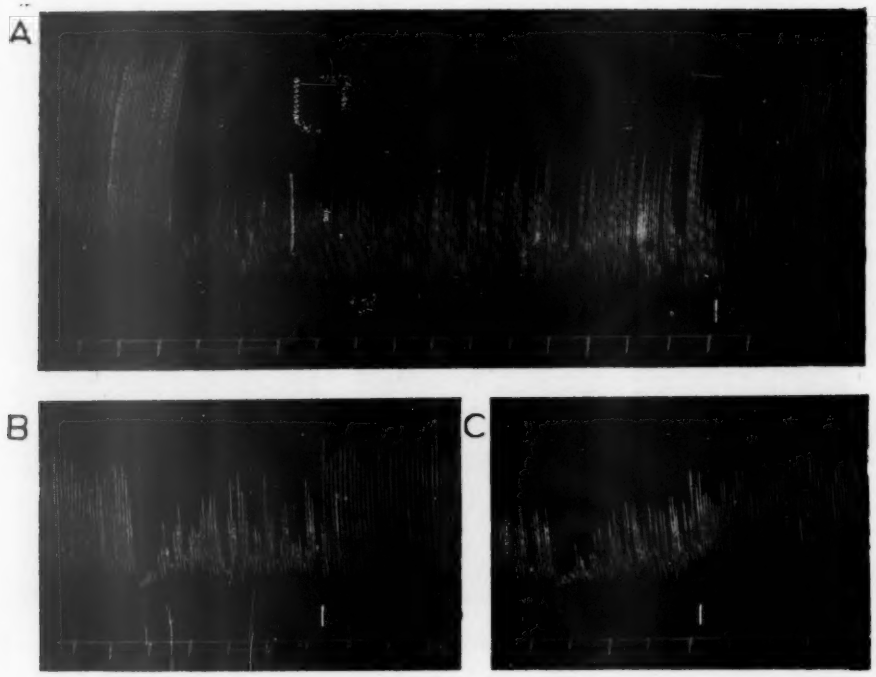

FIG. 3. — *Reversing action of the relaxing effect of adrenaline by a decrease in temperature* (A), *by azide 0.0005 N* (B), *and by the suppression of the bubbling of air* (C). *The vertical bars mark the time at which every one of those agents was tested. In this and in the following figures the time markings are one minute intervals.*

is being bubbled only with atmospheric air. In addition, the rate of shortening of a given fiber, the tone and the speed of conduction of an impulse from active to inactive fibers may also change with temperature. The end result is, that for the height of the recorded contractions, there is an optimal temperature. For the experimental conditions employed this optimum was about 30° to 32° C. The contractile activity appears clearly at about 18° C, and disappears near 45° C. Between the range of 25° to 35° C. there is not a significant change in the amplitude of the contractions.

B) *The effects of adrenaline.* — Adrenaline induces an increase in the rate of oxygen consumption of the isolated intestinal segments. The maximum of the effect was reached after 10 to 20 minutes from the moment it is added to the solution. For the concentration of adrenaline used (0.1 μg per ml) the increase was of about 300 per cent of the basal value. This effect usually persisted after replacing the solution by pure Ringer's.

The effect of adrenaline on the mechanical responses is the well known inhibition of the tone, together with a reduction of the amplitude of the rythmic contractions. Electrical activity is not inhibited during the period in which mechanical events are abolished, as has been previously described (Alvarez and Mahoney, 1922; Berkson, 1933). Both in the mechanical and in the electrical records it can be seen that the frequency of the oscillations increases in about 10 to 30 per cent under the influence of adrenaline (figs. 2 A and 6).

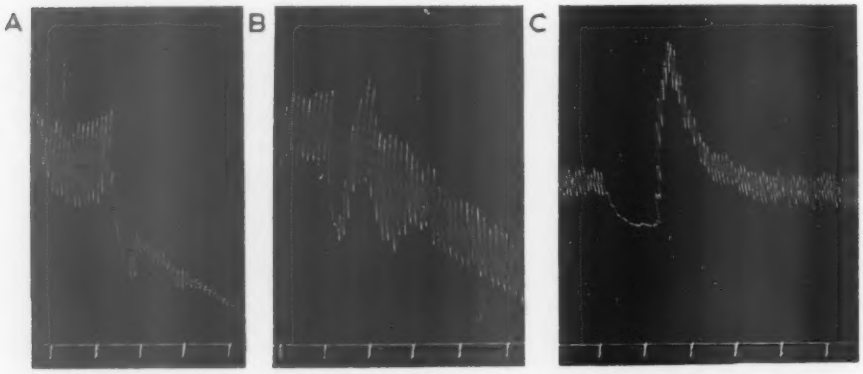

FIG. 4. — *Gradual appearence of the stimulating effect of adrenaline with time. Same dose of adrenaline in every case, 2 micrograms in 20 millilitres.* A. *One of the first responses recorded. The relaxation lasted for about 15 minutes.* B. *Two hours after* A. *The relaxing effect was less marked and less lasting.* C. *30 minutes after* B.

The duration and magnitude of the inhibition of mechanical activity is variable. They are greater and last longer at a lower temperature than at a higher one. Roughly, the logarithm of the duration varies linearly with temperature and, for the range explored, it showed the same kind of relation as the one between the rate of oxidation of adrenaline and the temperature.

The duration of the period of inhibition depended also on the amount of oxygen present in the solution at that moment. The inhibition was less, the lower the amount of oxygen made available to the preparation according to its actual needs. Reduction of the oxygen supply during the period of inhibition may result in an increase in the amplitude of the movements (fig. 3 C), which sometimes reaches heights as great or larger than the basal values before adding the adrenaline. However, if the lack of oxygen was prolonged the effect was a decrease of the mechanical and electrical activities, as is the usual response in a control preparation (fig. 5 A).

Repeated doses of adrenaline have a lesser augmenting effect of the oxygen consumption. The basal value increases since the very first dose, and it does not come back to the original value but very slowly (two

hours), even after the solution is replaced by pure Ringer's. Therefore, the decrease in the magnitude of the effect is in part due to a high basal value present before each successive dose. Similarly, the inhibition is less after the first dose, and also decreases with repeated doses, particularly if the preparation is left in contact with the adrenaline for longer periods of time, or when the preparation is tested after repeated washings (fig. 4), or when it has been on a reduced oxygen supply or even if it is taken from the animal after many hours from the start of the experiment. It is interesting to point out that all these conditions are also the ones in which a small dose of adrenaline can produce the opposite

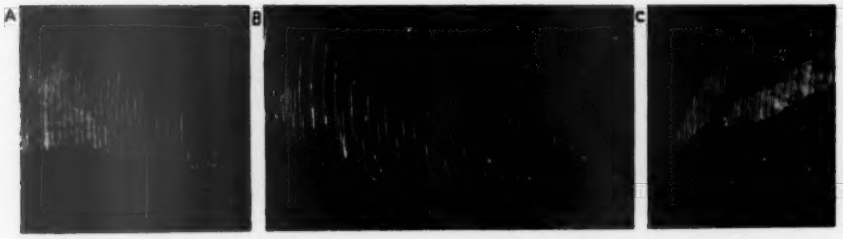

FIG. 5. — A. *Shortly after the start of the record the bubbling of air was stopped. The effect was fully reversible.* B. *Behaviour of an "inverted" preparation. At the end of the record the intestinal segment was brought back to its original position, with the mucosa inside.* C. *Shows the recovery of that same preparation. The vertical bars mark the moment at which adrenaline was added to the solution, in every case.*

response, that is, an increase in tone and in amplitude of the contractions. This stimulating effect was always observed to come after a small and transient inhibition.

The mechanical effects do not have a temporal course parallel to the increase in oxygen consumption, or to the rate of oxidation of the adrenaline in the solution. In fact, the rate of oxidation of this substance was found to be of the same order of magnitude as when it occurs *in vitro* with no living tissue present. It depends on the temperature, but is always much slower than is indicated by the course of the relaxing effects. Furthermore, the already apparently inactive solution in which and intestinal segment has been immersed may show good activity when tested on another preparation. The response of this second preparation had the same characteristics as those seen in the first one.

C) *The effects of adrenaline as modified by different agents.* — In the preceding section it was shown that a change in the temperature or in the oxygen tension of the solution may affect the responses to adrenaline. The metabolic inhibitors: cyanide or azide may also block the relaxing effects of adrenaline on intestinal smooth muscle. The action on the frequency seems more sensitive than the one on the amplitude. A concentration of 5 mM of cyanide or azide was enough to suppress the effect on the frequency. In order to block the effect of adrenaline on the amplitude of the responses a concentration of these inhibitors one hundred times greater was necessary. Still higher concentrations of these inhibitors,

particularly cyanide had a relaxing as well as a slowing effects of their own.

If the inhibitory response to adrenaline is already in progress, an effective dose of cyanide or azide can reduce considerably the magnitude of the effects of that substance. The amplitude of the contractions may reach the height of the control values, the frequency being lower (fig. 3 B). A similar result was obtained if at that moment, instead of adding the metabolic inhibitor, the preparation was suddenly cooled (fig. 3 A). We stated before that the same thing happens when the oxygen tension of the solution is reduced (fig. 3 C).

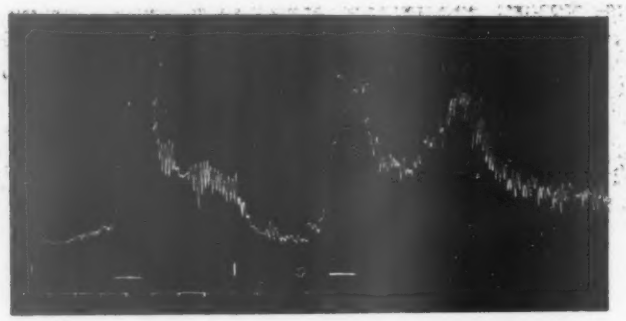

FIG. 6. — *Comparison between the effects of adrenaline (2 micrograms in 20 millilitres), and that of lactic acid (4 micrograms in 20 millilitres) added at the second vertical bar. The horizontal bars mark the time at which the solution was changed by pure Ringer's. Note the increase in the frequency of the contractions with adrenaline, and not with lactic acid, and the delayed increase in tone after the latter.*

At this time it will be convenient to describe the behaviour of the "inverted" preparation. The observation after the inversion has to be made as soon as possible in order to see some mechanical activity. If this is obtained, it is seen that the amplitude and the frequency of the movements gradually tend to decrease, eventually reaching zero. If at that moment, the preparation is reinverted, that is, brought back to its original condition with the mucosa inside, the movements gradually recover, both in size and frequency. This behaviour is similar to that of a control preparation to which the bubbling of air is suppressed. The recovery in both cases is fully reversible (fig. 5).

D) *The effects of lactic acid.* — As shown by Mohme-Lundholm (1953) lactic acid has an effect very similar to that of adrenaline. A concentration of 0.2 μg per mililiter has about the same relaxing action as 0.1 μg per mililiter of adrenaline. There are however some differences. Adrenaline increases the frequency of the contractions (figs. 2 and 6), lactic acid has a slowing effect. After removing adrenaline from the bathing solution the recovery is almost complete and instantaneous. The effects of lactic acid are less easily reversible, particularly to a high dose, or when it has been in contact with the preparation for a long period. After removing lactic acid, a short time after

a moderate dose, there is an increase in tone (fig. 6). This last difference seems only quantitative because the increase in tone can also be observed after replacing a solution containing a high dose of adrenaline by pure Ringer's of the same temperature and oxygen content.

Both these substances did not have a significant effect on the size of the electrical potentials recorded. The frequency and amplitude of the electrical oscillations generally increases with adrenaline and not with lactic acid (fig. 2).

The effects of lactic acid may sum with those of the adrenaline. Added together, the relaxation was greater than the one seen when only one of those substances was added alone. A small dose of lactic acid less than 0.05 µg per mililiter, may have a stimulating effect. The tone and the amplitude of the contractions increase after a slight relaxing action. Several of these small doses may sum their stimulating action but not indefinitely. A time comes after which the relaxing effect becomes the more apparent change. This time depends on the total amount of lactic acid added.

E) *The effect of bicarbonate.* — Bicarbonate has an effect of its own. It increases the tone and the amplitude of the contractions. This effect is a transitory one, and may be followed by a depressing action. At the concentrations employed in this study, which was a quantity just enough to immediately bring the pH of the Ringer solution to 7.2 after lactic acid had been added in proportion of 0.2 µg per ml, bicarbonate added alone has only the stimulating action (fig. 7 B and E). The depressing effect that follows after a high dose seems to be associated with precipitation of calcium from the solution. The stimulating effect is less apparent in older preparations or in those taken from the animal late in the day of the experiment, it also decreases somewhat with successive doses.

Bicarbonate not only inhibits the relaxing response to lactic acid, but reverses it to a stimulating effect (fig. 7 D and E). After addition of bicarbonate, and once this solution had been replaced by pure Ringer's, lactic acid, in small amounts, has a greater stimulating action than in the case when bicarbonate has not been used previously (fig. 7 C). In this case the pH of the solution is lowered by the addition of lactic acid.

When lactic acid is added after bicarbonate, the stimulating effect persists longer that the neutralizing action on the solution. As the time passes, the pH increases at a rate of about two tenths of a unit per minute. This is because of the formation of sodium lactate and carbonic acid, with the subsequent loss of this acid as carbon dioxide. The stimulating action of bicarbonate is also greater when it is added after lactic acid (fig. 7 D). This effect occurs even if the dose of lactic acid is small, and the change can coexist with a high pH value. At the usual concentrations employed, bicarbonate alone brings the pH of the Ringer solution to 8.0.

The relaxing effect of adrenaline may not be completely abolished by the previous addition of bicarbonate, but was always greatly reduced, as was previously shown by Mohme-Lundholm (1953). When bicarbonate is added during the relaxing response to adrenaline it can produce a slight increase in tone and in the amplitude of the contractions

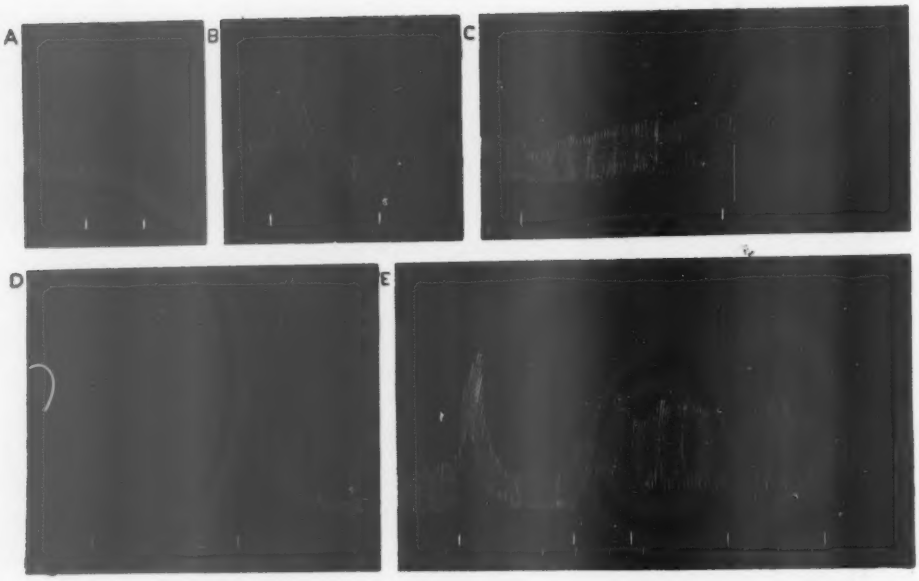

FIG. 7. — *The effects of adrenaline and of lactic acid as modified by bicarbonate.* A. *Bicarbonate added, at the second bar, during the relaxing action of 0.1 microgram of adrenaline per millilitre.* B. *Adrenaline tested (second bar) after bicarbonate (first bar).* C. *Lactic acid added in small amounts. At each bar, 0.05 micrograms per millilitre were added to the solution. After the first addition, the pH of the outside solution was lower than the control.* D. *Bicarbonate added during the relaxing effect (second bar) of 0.2 micrograms per millilitre of lactic acid.* E. *After bicarbonate (first bar), several doses of lactic acid (0.2 micrograms per millilitre) were added at each signal. A short time after the first addition the pH of the outside solution was higher than the control. It became lower only for a short period after each succesive dose.*

(fig. 7 A). After smaller doses of adrenaline a complete recovery may be obtained with the bicarbonate, or even may appear a greater mechanical activity than in the control conditions. This depends more on the dose of adrenaline used than on the final concentration of bicarbonate.

DISCUSSION

Intestinal smooth muscle activity includes two different processes: automaticity and contraction. The first one appears to be essentially dependent on chemical changes. It has a high thermal coefficient (2.5), and is easily affected by low oxygen tensions (fig. 5), and by the metabolic inhibitors azide and cyanide. Adrenaline has a stimulating action on it (figs. 2 A and 6).

On the other hand, the agents studied which have an effect upon the oxidative chain of reactions, produce only small changes on the con-

tractile activity when they are applied during the basal conditions. Temperature variations, or changes of the oxygen supply, do modify the amplitude of the contractions, but this is done at a slow rate, and the change produced is not too great, excepting the case when this variation is large or prolonged. A similar statement can be made for the effects of cyanide or azide.

The behaviour of the "inverted" preparation might be explained as a result of the reduction of the oxygen supply to the smooth muscle. It has been reported that the intestinal mucous membrane has a very high QO_2 (Dickens and Weil-Malherbe, 1941), and it is possible that this membrane uses all the oxygen available when it is diffusing into the tissue. This idea is supported by the observation of the fast recovery seen after the intestine is brought back to its normal position.

The present observations support the idea that the contraction is not a phenomenon directly dependent on an active oxidative metabolism, and that oxidative reactions are more closely related to the recovery process. This idea has been amply accepted.

On the contrary, the responses to adrenaline seem essentially dependent on oxidative metabolism. Oxygen consumption is increased, and although it is clear that there is not a parallel temporal course between these two events: rate of oxygen consumption and mechanical changes, the fact is that any factor which interferes with oxygen utilization rapidly affects the mechanical response to adrenaline. One might reasonably conclude that such a response is essentially dependent, directly or indirectly, from oxidative reactions.

It is not easy to see how the increased oxidative metabolism may interfere with smooth muscle mechanical activity. Cori and Cori (1931) showed that for rat skeletal muscle adrenaline increases glycogen breakdown through hexosemonophosphate to lactic acid, and subsequent oxidative removal of this lactic acid. From the observations of Lundholm and Mohme-Lundholm (1957) it seems that the same type of effect occurs in smooth muscle. According to these authors, lactic acid derived from the accelerated glycogenolysis would be the responsible for the relaxing action. It is possible also that lactic acid be the responsible for the increase in oxygen consumption. This would be a good explanation and seems to be supported by the available information (Mohme-Lundholm, 1953 and 1957). The reduced effects of adrenaline on the mechanical responses in old preparations would be associated with a depletion of the glycogen stores, the same is indicated by the lesser effect on the oxygen consumption (section B).

There are, however, some results which may bring some doubt on that simple mechanism: a) The relaxing effect of adrenaline has a shorter duration than the increase in lactic acid production, if this can be judged by the temporal course of the increase in oxygen consumption, or by the rate of oxidation of adrenaline in the tissue, which are by far slower and more prolonged processes. b) The relaxing effect of adrenaline, and probably not the lactic acid production, is abolished when the oxidative reactions are depressed by a low oxygen tension (figs. 3 and 5). c) Lactic acid does not have the accelerating effect of adrenaline on the frequency of the contractions (fig. 2).

It seems necessary, therefore, to make some additional hypotheses to cover those facts. First, that adrenaline, apart from its glycogenolysis accelerating action has a direct stimulating effect on impulse initiation. Second, that some other factor appears after lactic acid production is accelerated. This second factor would have a stimulating effect on the contractile system. Its production, or the rate at which it would be inactivated or destroyed would be affected by the agents which interfere with oxydative metabolism. The present observations give a basis to establish the existence of this second factor. It may be associated or identified with a high pH value inside the muscle, derived from the combination of lactate with the base of the cells.

This last hypothesis may be formulated in detail as follows: lactic acid produced by the glycogenolytic action of adrenaline would exist in a free form and through the increase in hydrogen ion concentration would produce the relaxation of muscle. But slowly it would be converted into lactate by displacing the bicarbonate of the alkaline reserve of the cell, with the production of carbon dioxide. In this form, by itself, or rather by increasing the intracellular pH it would be the responsible for the stimulating action. Added bicarbonate would act by its combination with the lactic acid present in the cell, and also it would increase the alkali reserve. This would explain its antagonism with the relaxing effect of adrenaline, also reported for it and for ammonium carbonate by Mohme-Lundholm (1953). Lactic or acetic, or perhaps also any other diffusible organic acid, when added after bicarbonate would combine inside the cell or would diffuse into it as the base combined anion. In that form they would have their great stimulating action (fig. 7 D and E). When lactic acid is present in high quantities inside the cell, the stimulating action of bicarbonate has to be greater according to this theory (fig. 7 D), excepting the case in which lactic acid is being continuously produced, as is the case of the action of adrenaline. The stimulating action of bicarbonate is in fact very small when it is added during the first minutes of the relaxing effect of adrenaline (fig. 7 A).

It remains to explain the stimulating action of adrenaline in a several hours old preparation, or in one in which oxidative metabolism is depressed. For the first of these cases, it might be that the glycogenolysis occurs then at a slower rate. There is some fundament for the idea that the glycogen stores must be depleted with time, and possibly too in the case in which the intestine has been kept in the anesthetized animal for a long period. This is necessarily a fasting period for the whole animal. In this kind of preparations we reported also that bicarbonate has a reduced effect, and this also suggests that the amount of lactic acid produced is going down. For the second of these cases, we have two possibilities. Lundholm and Mohme-Lundholm (1957) explained the blocking effects of enzyme inhibitors as an interference with the glycogenolysis accelerating action of adrenaline. The same type of explanation can be used here for the reversing action of cyanide or azide, an also for the effect of lowering the temperature. The similar effect seen after the reduction in the oxygen supply, however, does not easily accept that same explanation. Hegnauer and Cori (1934) showed that lactic acid production by adrenaline was greater in frog muscle kept under anaerobic conditions. This observation might not be applicable to intes-

tinal smooth muscle, although this does not appear very sound in view of the many other existing analogies. A second alternative hypothesis would be that the removal of lactic acid through oxidation, by being slower, would favor its combination with the alkali reserve of the cell, with the consequent appearance of the stimulating action of the resultant lactate. This idea is compatible with the present results, and it also could be applied to explain the effects of the other agents mentioned above, which are well known as inhibitors of the oxidative reactions of the tissues.

Still, a possibility is that adrenaline, by itself, is the stimulating factor, and that this action requires a pH value at the alkaline side. This possibility would not explain the effect of the bicarbonate alone.

The above discussion does not mean to indicate that the relaxing action of lactic acid or the stimulatory effect of lactate are exerted directly on the contractile mechanism. Contraction and relaxation are due to vastly complex mechanisms, and the action of these substances, although of a general character on different types of smooth muscle, must not be considered as a primary one. They would act favoring or depressing some steps in the chain of reactions from which the corresponding energy is derived. The exact nature of this influence remains unexplained.

SUMMARY

On isolated segments of the small intestine of the rabbit the effect of temperature, low oxygen supply, cyanide, azide and bicarbonate were studied upon the electrical and mechanical activities. The same agents were tested on the responses to adrenaline and to lactic acid added in fixed quantities.

The results obtained support the idea that rythmicity is a phenomenon directly dependent on oxidative metabolism. Contractility, on the other hand, only indirectly is affected by the changes in the rate of tissue oxidations. Rythmicity is easily affected by temperature changes and by the agents that interfere with metabolism. It has a high thermal coefficient (2.5), it is depressed by low oxygen tensions, by cyanide and azide (figs. 3 and 5) and it is enhanced by adrenaline (fig. 2).

It was found that adrenaline greatly increases the rate of oxygen consumption of this tissue. It was confirmed that the relaxing effect of adrenaline is intimately related to its accelerating action on lactic acid production. Added lactic acid had a similar effect (figs. 6 and 7). Although this acid lacks the stimulating effect of adrenaline on rythmicity (fig. 2).

The relaxing effect of adrenaline has a shorter duration than lactic acid production, the latter being judged by the temporal course of the increase in oxygen consumption or by the rate of oxidation of adrenaline in the tissue, both of which are more long lasting effects. This is explained as be due to the lactic acid combination with the base existing in the cells, and to the stimulating action of the resultant alkaline lactate.

This hypothesis is supported by the observed effects of added bicarbonate, which would increase the alkaline reserve of the tissue. Bicarbonate has a stimulating action (fig. 7 D). It reduces the relaxing effect of adrenaline (fig. 7 A and B), and reverses completely the same kind of

effect which is produced by added lactic or acetic acid (fig. 7 E). Lactate would be the responsible of the stimulating effect of small doses of adrenaline which is seen in a several hours old preparation (fig. 4). In this last case lactic acid production would occur at a slower rate than lactate formation. The slower rate of lactic acid formation would depend on the depletion of the glycogen stores. The inversion of the relaxing effect of adrenaline by the agents which interfere with oxidative metabolism (cyanide, azide, cooling, low oxygen tension) (fig. 3), would be due to a favoring action on lactate formation, because of the slower rate of removal of lactic acid through oxidation.

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THE NEUROSECRETORY MATERIAL OF THE RAT'S INFUNDIBULAR PROCESS IN THE DIABETES INSIPIDUS INDUCED BY AMINOPTERIN

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LEWIS et al. (1952 a, 1952 b) observed that rats treated with either a single dose (near L. D. 50) or 5 μg/100 g daily of an antifolic (Aminopterin) showed a diabetes insipidus like syndrome. In these early studies the antidiuretic hormone was not investigated in the *pars nervosa* of the hypophysis and the possibility of a central origin was taken into consideration.

Rabasa and Bergmann (1954) demonstrated later the peripheral origin of this syndrome, which they showed was due to a lack of sensitivity of the renal tubule to the hormone, while the posterior lobe and serum of these animals assayed a normal antidiuretic activity.

The present work aims at the study of the neuro-hypophysis of these rats with Gomori's method (1941). The available experience warrants the use of this technique for the histological assessment of the "neurosecretory" material.

METHODS

Thirty rats weighing about 200 g were used.

They were divided into three groups: a) control group made up of 5 males and 5 females; b) 5 males and 5 females with diabetes insipidus obtained by a daily injection of 5 γ/100 g of Aminopterin and c) 5 males and 5 females deprived of water during 8 days. This treatment was used to check the method because it is known that water withdrawal causes a release of antidiuretic hormone from the posterior lobe.

The animals were killed by a sudden decapitation to avoid the discharge of hormone by painful stimulus. The rats with diabetes insipidus were sacrificed on the second day of the appearance of this syndrome.

The hypophysis were fixed in Bouin, included in paraffin, cut at

* This work has been done during the tenure of a scholarship of Asociación Argentina para el Progreso de las Ciencias.

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five microns on the sagittal plane and stained by the chrome hematoxylin phloxyn method (1941).

The quantity of "neurosecretory" material was measured by a score from 0 to 10, assigned by an observer who ignored the treatment, and was based on the amount of granules stained by the chrome hematoxylin. A square root transformation was used to normalize these data which were afterwards subjected to an analysis of variance segregating the observer's error in order to measure the consistency of the results.

TABLE I

Analysis of variance of the scores assigned by the observer

Sources	D. of F.	Sum of squares	F
Items	30	0.5369	
Sexes	1	0.001	
Treatments	2	15.222	19.7
Interaction	2	0.018	
Animals	24	9.302	21.5

$F_{0.01}$ (2 and 24 D. of F.) = 5.61.
$F_{0.01}$ (24 and 30 D. of F.) = 2.47.

RESULTS

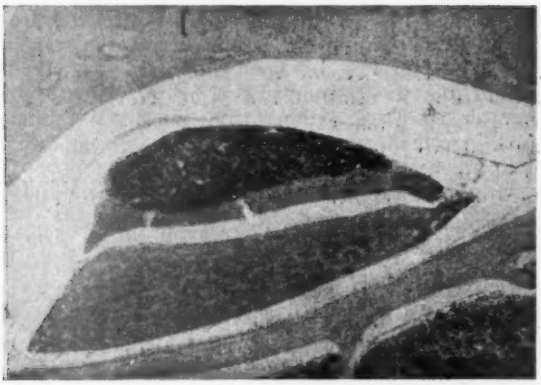
The mean scores (square root) are: normal, 2.61; dehydrated, 1.70 and Aminopterin treated rats, 2.88.

The analysis of variance (table I) shows that the observer is able to discriminate the animals within treatments ($F = 21.5$, $p < 0.01$), on which lies the validity of the experiment. No difference exists between normals and rats with diabetes insipidus due to Aminopterin, while the difference of these two groups and the dehydrated one is very significant ($F = 19.7$, $p < 0.01$).

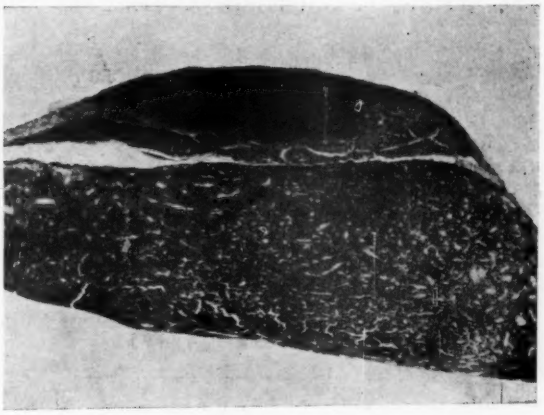
DISCUSSION

Since Bargmann (quoted by Rothballer, 1953) applied Gomori's method to study the neurosecretion in the dog, a histological technique is available to study the correlation of the physiological states of the posterior hypophysis with its morphology. The value of this method was confirmed in other species by Smith in rodents (1951), Scharrer in insects (1951) and Palay in man (1952) (quoted by Rothballer, 1953).

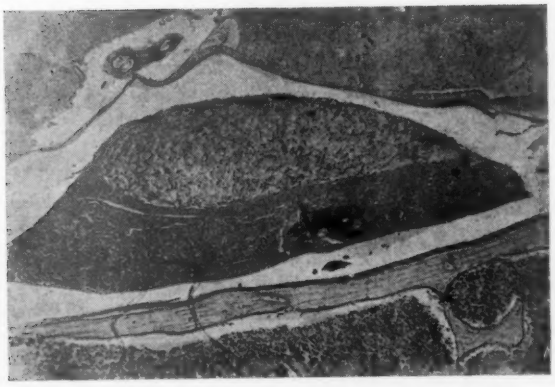
Another contribution to this correlation was that of Scharrer (1952) (quoted by Rothballer, 1953) who observed the disappearence of the granules with the dehydration and its reappearence with the intake of water.



1) Pituitary gland of normal rat. A great quantity of black granules can be seen in the pars nervosa.



2) Hypophysis of a rat with diabetes insipidus due to Aminopterin. The amount of the neurosecretory material is similar to the normal.



3) Pituitary gland from a rat deprived of water during 8 days. There is a depletion of neurosecretory material.

PLATE 1. — *Sagital sections of the rat's hypophysis (5γ) stained with chrome alum hematoxylin. X 50.*

Our results show that this method is able to detect a difference when a discharge of hormone is known to exist (water deprivation) (fig. 3) so that the lack of difference between normals (fig. 1) and rats with diabetes insipidus due to Aminopterin (fig. 2), is consistent with Rabasa and Bergmann's findings (1954) that these animals have a normal quantity of antidiuretic hormone. Conversely, since this was the expected result it can in turn be considered as another proof of the close correlation existing between the substance so stained and the antidiuretic hormone.

Summary

The histological study of the neurosecretory material of the rats infundibular process shows no difference between normals and rats with diabetes insipidus due to Aminopterin. This fact confirms the view that the rats with diabetes insipidus induced by Aminopterin have a normal quantity of antidiuretic hormone.

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THE GLYCOGENOLYTIC ACTION OF ADRENALINE AND NORADRENALINE ON THE ISOLATED LIVER OF THE TORTOISE

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SINCE Blum [1] showed in 1901 that the injection of adrenaline produced hypoglycemia, many reports have been made on the influence of this hormone on the glycogenolytic function of the liver.

It is always interesting to study the problem of the regulation of the glycemia in various animal species. For this reason we accepted the suggestion made by Dr. Ricardo R. Rodríguez to study the glycogenolytic action of the hormones of the adrenal medulla on the isolated liver of the tortoise, as done previously by Képinov [2, 3, 4, 5] and Houssay and Gershman [6, 7] in batrachians.

MATERIAL AND METHODS

In the experiments to be described, performed between July and December, the livers of 47 tortoises were used, 37 females of the genus *Chrysemys d'Orbignyi*; the other 10, 3 males and 7 females, were of the genus *Phrynops Hilarii*. Some of the animals were used immediately after they were captured in their natural environment and the others remained (for variable periods) in a special pool and were daily fed mainly on fish.

The liver of each animal was used for only one experiment and was prepared according to the following method:

The animal was insensibilized by the section of the spinal cord at the height of the occipito-atloid articulation; it was then restrained on a special board with the shell upward, with the limbs and the neck stretched by means of strings. An U shape window was made on the back with a saw, taking good care of the deepness of the cut, once the inner table was passed, to avoid any damage of the liver and blood vessels specially of the pectoral girdle. Once the rectangle of shell was removed the superficial abdominal veins were dissected and sectioned between two ligatures. The same was done with the arteries and veins that supply

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the head and the upper limbs. The celiac artery and the hepatic porta and inferior vena cava were also sectioned in the portion near the urinary bladder after the latter was emptied. The esophagus, the trachea and the rectum were then sectioned and the heart, the lungs, and the remaining digestive tract with its corresponding glands were removed in only one block by "decolage". These viscera were placed in a dish containing Ringer's solution. The object was to avoid the desiccation of the liver, to permit manipulation and to avoid breakage of the hepatic tissue by adherence to the dish. In the tortoises of the genus *Chrysemys* as the right lung is united to the hepatic lobe of the same side, it is necessary to isolate it; this is done by dissection of the two organs through a cleavage plane. Then all the vessels that supply the liver were ligated except the hepatic portal and the two suprahepatic veins which were cannulated. The perfusing fluid entered through the hepatic portal and was collected through the other two veins.

As the hepatic portal reaches the liver after passing through the pancreas, this vessel was cannulated in order to permit the removal of all the pancreas, so that during the perfusion no pancreatic hormones could pass to the liver together with the injected adrenaline and noradrenaline. The bile duct was also dissected and ligated.

As it was difficult to find livers of the same weight, they were distributed in groups as homogeneous as possible. At the beginning of the experiments the perfusion was done at room temperature, but as the results of preliminary experiments proved the importance of the temperature, the perfusion was then performed in a chamber at 27° C ± 1° C.

Once the liver was placed in the chamber, covered with gauze soaked in Ringer's solution it was not touched more because it was observed previously that any manipulation of the organ produced greater liberation of glucose in the perfusing fluid.

The perfusing system was composed of two communicant vessels at constant pressure, mantained at a height of 75 cm from the liver, and provided with a mechanism which allowed the maintainance of the volume of the collected fluid (about 80 ml in 30 minutes) constant up to a certain degree. It was also possible to pass from one to another perfusion without interrumption of the flow.

Every liver was perfused first for one hour and 30 minutes with Ringer's solution which was discarded. The object of this washing was to free the organ of blood, glucose or other substances that could influence glycogenolysis. Then the perfusion proper was started, which lasted 4 and ½ hours, was measured and the sample was collected for glucose determination. The method used for glucose determination was Somogyi-Nelson.

The Ringer's solution used had the following composition, for 100 ml: NaCl, 0.65 g; KCl, 0.01 g; $NaHCO_3$, 0.02 g; $CaCl_2$, 0.02 g; Na_2HPO_4, 0.005 g.

The solutions of l-adrenaline and l-noradrenaline were prepared from stock solutions of 1 g per liter which were diluted in Ringer's to obtain the desired concentrations and 10 mg % ascorbic acid was added to avoid oxidation. These solutions as well as the Ringer's were prepared immediately before use.

RESULTS

The livers perfused only with Ringer (controls) showed a progressive decline of their spontaneous glycogenolysis as long as the perfusion progressed, but never attained a 0 value even when the livers were perfused for more than 12 hours.

Adrenaline in dilutions of 1:500 000 to 1:300 000 000 added to the Ringer produced a definite rise of the spontaneous glycogenolysis which

TABLE I

Concentrations of adrenaline and noradrenaline and glycogenolysis

Concentrations	Nº of cases	Temperature	Ringer	Ringer	Ringer
Controls	7	19 - 21 - 27	100 99 96	101 95 92	87 87 87
			Ringer	**Adrenaline**	**Ringer**
$.2 \times 10^{-8}$	3	19 - 21 - 27	100 84 70	82 84 95	97 96 101
$.3 \times 10^{-8}$	2	27	100 97 119	128 160 146	130 120 111
$.5 \times 10^{-8}$	5	19 - 22 - 27	100 101 98	118 157 163	182 131 107
1×10^{-8}	4	16 - 18 - 27	100 131 131	175 262 271	254 194 154
$.5 \times 10^{-7}$	2	12 - - 14	100 79 69	170 236 317	291 247 201
1×10^{-7}	2	27	100 74 67	137 259 330	291 232 153
$.2 \times 10^{-5}$	3	27	100 93 92	228 390 415	343 283 225
			Ringer	**Nor-adren**	**Ringer**
1×10^{-8}	5	27	100 105 108	104 126 128	128 117 117
1×10^{-7}	5	27	100 94 97	111 170 211	170 131 108
1×10^{-6}	4	27	100 130 101	219 308 324	297 217 191
1×10^{-5}	5	27	100 107 109	236 363 383	224 254 148

Figures represent per cent the mean values for glycogenolisis in every period of 30 minutes of perfusion. The increase in glycogenolisis was calculated considering as 100 % the amount of glucose obtained in every perfusion during the first half hour; the results of the following periods are refered to this cipher.

in most cases reached a maximum during the third half hour of perfusion with the drug. This rise was transitory because it gradually disappeared when the addition of the sympathicomimetic amine was stopped. Lower concentrations as 1:500 000 000 for instance were ineffective.

Noradrenaline also had a definite effect with dilutions of 1:100 000 and 1:10 000 000. Also with noradrenaline the effect was transient and the maximum was noted on the third half hour of perfusion with the drug. Concentrations of 1:100 000 000 were ineffective.

The results are summarized in table I.

Figure 1 shows the relation between the glycogenolysis of controls and that of perfusion with adrenaline and noradrenaline at different concentrations. The general course of each perfusion whether with Ringer, noradrenaline or adrenaline is also shown.

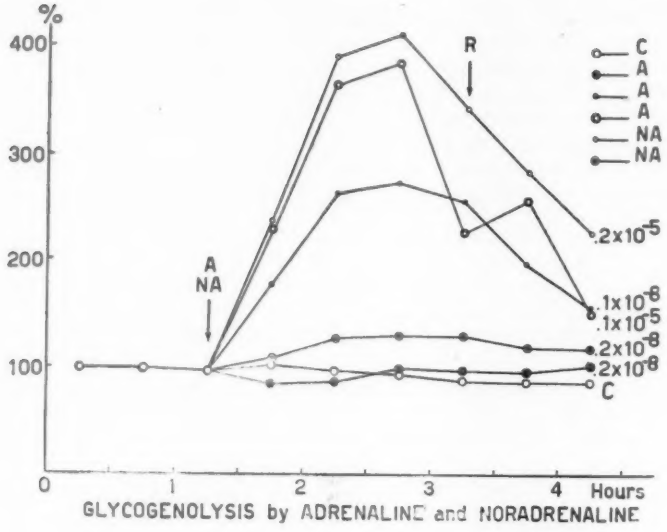

FIG. 1

DISCUSSION

The amount of glucose obtained in these experiments represent the maximum capacity of production of glucose by the perfused liver. No glucose was present in the perfusion fluid nor any other substances capable of reducing the Somogyi-Nelson reagents which, as it is well known, are not specific for glucose.

The following factors should be considered:

a) *Animal species and sex.* — As to the glycogenolytic response, neither the animal species *(C. d'Orbignyi and P. Hilarii)* nor the sex had any influence since the amount of glucose liberated from the livers were similar in our experiments. For this reason results in animals of different species and both sexes were put together in some groups.

b) *Oxygenation.* — Our experiments were done without oxygenation of the perfusor fluid. As glycogenolysis is an anaerobic mechanism we think that the lack of oxygen did not greatly influence the results. A definite answer, however, can only be given when perfusions with and without oxygen are performed.

c) *Rate of the perfusion.* — The material used did not allow a definite conclusion concerning this factor. But, our data allow us to conclude that, within certain limits, the production of glucose by the hepatic cells does not depend on the volume of fluid perfused. On the other hand it does depend on the concentration of glucose of the fluid. Moreover, this is in agreement with the ideas of Soskin ([8], [9]) that the level of glycemia determines the hepatic response.

In future experiments we shall study to this point.

d) *Temperature.* — As early as in 1947, Képinov ([10]) in studying the mechanism of the seasonal variations of the hepatic glycogenolysis in frogs calls attention to the influence of this factor not only the transformation of glycogen to glucose but also on the inverse process. In a series of experiments he shows that when the animals are placed at low temperature a reduction of glycogenolysis occurs. He attributed this effect to an influence of the anterior lobe of the hypophysis: in the first case there would be a decrease of a hormone which he called "glycogenotropic", and consequently a less intense glycogenolysis. In the second the inverse mechanism would be observed.

TABLE II

Spontaneous glycogenolisis and temperature

Weight of liver	Temperature	Glucose/min/g. of liver
92 g	15 °C	7 µg
95 g	27 °C	16 µg
100 g	13,5° C	4 µg
100 g	27 °C	19 µg
120 g	13,5° C	3 µg
133 g	27 °C	12 µg
174 g	12 °C	4 µg
160 g	27 °C	26 µg

The findings reported here when perfusions were done at low temperature agree and confirm absolutely the observations of Képinov, as can be seen in the following table II, in which the spontaneous glycogenolysis of livers of similar weight, expressed as µg of glucose/minute/g of liver at different temperatures are presented.

These comparative values are more demonstrative if we remember, as was also proved, that a lineal relationship exists between the weight of the livers and the amount of glucose produced by them.

The temperature of 27° C was chosen because it was the mean rectal temperature of the animals in captivity during the warm months of the year.

Perhaps it will seem strange that in spite of the above we include in table I results of perfusions performed at low room temperature. This apparent contradiction is due to the presence of a normal glycogenolysis in those livers, a fact that is explained because the fall of the ambiental temperature was too rapid to permit hypofunction of the anterohypophysis as was mentioned before.

We have not studied in these experiments the higher and lower limits

of temperature that influence glycogenolysis. This might be the object of future research.

After these general considerations, we may analyse the glycogenolytic effect of adrenaline and noradrenaline. Both drugs produce on the isolated perfused liver a definite glycogenolytic effect, more intense in the case of adrenaline (ineffective only at 1:500 000 000) and less strong for noradrenaline (ineffective at 1:100 000 000). The effects of the drugs are related to the concentrations used, as can be easily observed in the following table III were the value for b (slope of the regresion line)

TABLE III

Relation between the values of b and the concentrations studied

ADRENALINE		CONCENTRATIONS	NOR/ADRENALINE	
with drug	washing		with drug	washing
7.7	1.7	$.2 \times 10^{-8}$		
11.3	— 9.7	$.3 \times 10^{-8}$		
23.4	— 21.9	$.5 \times 10^{-8}$		
50.7	— 41.0	1×10^{-8}	8.2	— 4.4
81.0	— 39.2	$.5 \times 10^{-7}$		
90.1	— 59.5	1×10^{-7}	40.1	— 34.8
		1×10^{-6}	71.6	— 47.9
131.1	— 63.0	$.2 \times 10^{-5}$		
		1×10^{-5}	94.9	— 67.5

b was calculated by means of the equation $y = a + bx$ that is resolved by the following equations: $na + \Sigma bx = \Sigma y$, and $\Sigma ax + bx = \Sigma xy$.

is given for the different concentrations, during periods of perfusion with the drugs and during the washings.

The lower potency of noradrenaline was observed already by Sahyun in 1933, by McAuliff in 1949 and by Ellis ([13]) in 1951. The two last authors found 1/8 and 1/5 of the potency of adrenaline. Our results agree better with those of the last author and with those reported by Williams ([12]).

Another important peculiarity is the great sensitivity of the liver of the animals used. Houssay and Gershman ([7]) have already observed a greater sensitivity on this aspect, of *Bufo arenarum* Hensel in comparison with the european frog. In our animals the sensitivity is even greater, through we can not make a strict comparison with the experiments performed by Képinov and by Houssay and Gershman, because the first made only one injection of adrenaline and the second two authors did not specify the temperature at which the experiments were done.

The values of b in the washings also show a greater fugacity of the effect of adrenaline when compared with noradrenaline [less intense destruction by aminooxidase — Williams ([12])].

The maximum effect of both drugs, observed at the third half hour from the beginning of the perfusion, was always present at this very period, even when the perfusion was more prolonged and for this reason we limited the experimental time to 1 ½ hours of perfusion with adrenaline and noradrenaline. This decline of the glycogenolysis in longer perfusions can not be explained by the absence of hepatic glycogen because when the perfusion ended and glycogen was determined in the liver, it was found in appreciable amounts. Also here our findings agree with Képinov's data, who explains this fact as the decrease in the liver of the glycogenotropic hormon of pituitary origin, which is necessary for the fixation of adrenaline on its target site of action.

SUMMARY

1) A technique for the perfusions of the isolated liver of the tortoise *Chrysemys d'Orbignyi* and *Phrynops Hilarii* is described.

2) The glycogenolytic effect of adrenaline is observed in these conditions in concentrations of 1:500 000 000 to 1:300 000 000.

3) The same effect is observed under the same conditions for noradrenaline in concentrations of 1:100 000 to 1:10 000 000.

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A SINGLE INJECTION METHOD FOR ESTIMATION OF SPLANCHNIC BLOOD-FLOW WITH B. S. P.

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IN 1945 Bradley et al. (1) introduced a method for estimation of splanchnic blood-flow based on constant infusion of bromosulphalein (BSP) and determination of its extraction by the liver using hepatic vein catheterization. Since then, several papers (2, 3) have been published using this method for the study of liver blood-flow in different pathological conditions.

In this paper we present a method for estimation of hepatic blood-flow based on a single injection of BSP.

METHODS

The procedure was carried out 15 times in a group of 9 patients free of clinically detectable cardiac or liver disease.

Patients were studied after 12 hours of fasting and after at least 30 minutes rest in recumbency. A cardiac catheter was introduced into an arm vein and advanced into an hepatic vein, usually the one draining the right lobe of the liver. An indwelling needle was introduced into a brachial artery for blood sampling.

A 5 % solution of BSP was injected in less than one second through a needle or a polyethylene catheter placed into an arm vein. The amount of BSP injected in each run, estimated by weighing the syringes before and after injection ranged from 1.52 to 3.3 mg/kg of body-weight.

Arterial blood samples were taken every two seconds starting on the 5th second after injection up to the 35th or 40th second for estimation of systemic blood-flow by the dye dilution method of Stewart-Hamilton (6). Starting the 6th minute, arterial and hepatic vein blood were drawn simultaneously every 2 minutes until the 20th minute.

In the same patients the complete procedure was repeated after 60 minutes, in three of them after breathing pure O_2 for 30 minutes

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before the second run started. Between the 6th and 20th minute an anaerobic blood sample was obtained simultaneously from the hepatic vein and brachial artery for the determination of O_2 content in a Van-Slyke manometric gas analysis apparatus.

The blood samples were collected in tubes containing dry heparin; the plasma was separated by centrifugation, diluted to three times its original volume and placed then in 3 ml Beckman cells. One drop of concentrated solution of sodium hydroxide was added and the BSP concentration was read immediately at a wave length of 5750 A by means of a Beckman Spectrophotometer model DU. The volume of packed red cells (Ht) was determined in arterial blood with Wintrobe tubes spun at 3.000 R.P.M. for 30 minutes. When plotting BSP plasma concentration against time on semilog paper (fig. 1) a straight line decay is obtained which may be extrapolated to zero time indicating the concentration at that time if the dye were mixed evenly and instantaneously in the distribution space, independently of the extraction by any organ or tissue ([8]). The BSP space (S_{BSP}) is estimated applying the following equation:

$$S_{BSP} = \frac{I \times 1000}{Co}$$

where I = amount of BSP injected in mg and Co = BSP concentration in mg ‰ at zero time. Since BSP space of distribution is the plasma volume, blood volume (QB) would be:

$$QB = \frac{S_{BSP} \times 100}{100 - Ht}$$

To estimate the clearance of BSP concentration points one minute apart were selected in the disappearance curve (fig. 1) such as C_1 and C_2 and the mean concentration between them determined ($Cm = \sqrt{C_1 \times C_2}$) such that:

$$Cl_{p\,BSP} = \frac{S_{BSP}\,(C_1 - C_2)}{Cm}$$

Plotting on the same paper (fig. 1) the disappearance rate as obtained from arterial and hepatic vein samples and comparing two similar points, as refering to time, BSP extraction by the liver may be estimated:

$$E = \frac{Ca - ChV}{Ca}$$

where E = extraction of BSP.
ChV = BSP concentration in hepatic vein blood.
Ca = BSP concentration in arterial blood.

Knowing BSP extraction, liver plasma flow (EHPF) and liver blood-flow (EHBF) may be estimated from the clearance of BSP:

$$EHPF = \frac{Cl_{p\,BSP}}{E} \quad \text{and} \quad EHBF = \frac{EHPF \times 100}{100 - Ht}$$

From hepatic blood-flow and concentrations of O_2 in the artery (Ca_{O_2}) and in the hepatic (ChV_{O_2}), blood O_2 uptake by the viscera in

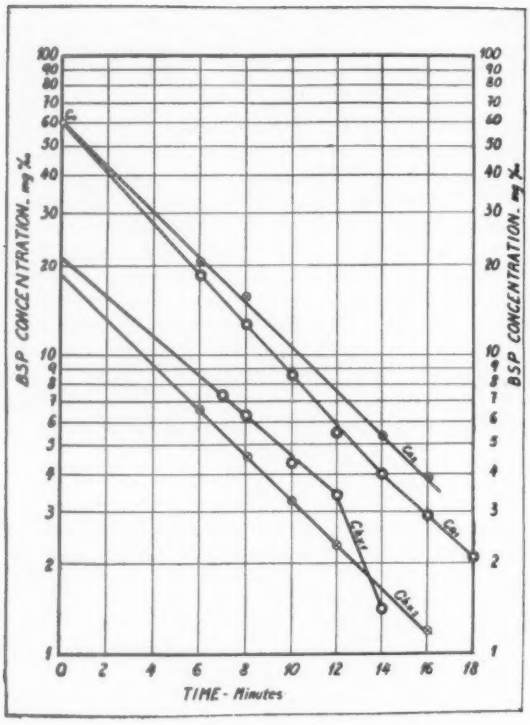

FIG. 1. — *Subject M. D. C.*

BSP: concentration disappearance curve.
Ca_1: concentration in arterial blood, first run.
ChV_1: concentration hepatic vein blood, first run.
Ca_2: concentration in arterial blood, second run.
ChV_2: concentration in hepatic vein blood, second run.
Co: extrapolated concentration at zero time. In this case the amount of BSP injected was the same for the first and second run.

the splanchnic area ($\dot{V}_2H$) may be computed:

$$\dot{V}_{O_2}H = EHBF\ (Ca_2 - ChV_2)$$

RESULTS

Results obtained are shown in table I. The mean plasma clearance of BSP was in 9 patients 607.11 ml/min ($\bar{\delta} = 233.71$ and $\delta_{\bar{x}} = 82.64$). In the 6 cases with a second run the mean $Cl_{p_{BSP}}$ was 565.5 ml/min ($\bar{\delta} = 238.6$ and $\delta_{\bar{x}} = 97.43$) so that the difference between means was 41.61 ml which was not statistically significant ($p = 0.722$). Extraction of BSP ranged between 39 to 96 %, mean 67.5 %.

Hepatic blood-flow ranged from 922 ml/min to 2843 ml/min, mean; 1702.9 ml/min ($\delta = 678.5$ and $\delta_{\bar{x}} = 226.1$). Comparing the results obtained in the same patients in whom two determinations are available the difference between means is 5 ml/min evidently not significant, as in three patients the second determination was carried out while breath-

TABLE I

Name	Age Y	Weight kg	Height m	B.S. m^2	Breath.	I_{BSP} mg/kg	S_{BSP} ml	QB ml	Cl_{BSP_p} ml/min	E	EHBF ml/min	Sa_{O_2} %	Ca_{O_2} Vol. %	$C_{VH_{O_2}}$ Vol. %	$\dot{V}_{O_2}H$ ml/min	PDR %	$\Delta A\text{-}V_{O_2}$ Vol. %
J.N. ♂	50	54.0	1.62	1.57	air	2.77	2112	3641	370	0.39	1653					17.7	
B.D. ♂	44	64.0	1.72	1.76	air	1.52	3478	6887	708	0.79	1773						
J.B.H. ♂	35	71.0	1.57	1.72	air	2.07	2588	4440	440	0.51	1500	92.0	17.44	13.01	66.4	17.3	4.43
A.Z. ♀	56	44.7	1.67	1.48	air	3.3	2200	3465	444	0.76	922					20.1	
						3.1	* 2200	* 3465	406	0.75	849					18.4	
A.B. ♀	25	53.4	1.56	1.51	air	2.61	2320	4142	518	0.96	957					22.3	
						2.64	* 2320	* 4142	357	0.60	1062					15.4	
V.G. ♂	23	73.5	1.75	1.88	air	2.13	4910	9340	1088	0.76	2728					22.2	
						2.15	4910	9430	806	0.57	2690					16.25	
M.D.C. ♀	54	62.0	1.52	1.59	air	2.47	2532	4275	517	0.63	1390	93.0	16.55	12.11	61.6	20.4	4.44
					O_2	2.35	2450	4065	427	0.70	1011	100.+.78	17.98	12.66	58.0	17.4	5.32
F.R. ♂	48	65.0	1.65	1.72	air	2.17	2083	3857	523	0.62	1559	90.8	18.57	13.67	76.5	25.1	4.90
					O_2	2.08	* 2083	* 3857	471	0.58	1501	100.+.40	20.81	15.11	85.4	22.6	5.70
A.O. ♂	44	62.0	1.58	1.63	air	2.95	4124	7403	856	0.54	2843	91.4	16.55	11.77	134.2	20.0	4.72
					O_2	2.21	3817	7202	926	0.50	3256	98.5	18.13	13.72	143.3	24.0	4.41

I_{BSP}: amount of BSP injected.

Cl_{BSP_p}: plasma clearance of BSP.

EHBF: estimated hepatic blood-flow.

$\dot{V}_{O_2}H$: oxygen consumption by the liver.

Sa_{O_2}: arterial oxygen saturation of hemoglobin.

S_{BSP}: BSP space. QB: blood volume.

PDR: percentage disappearance rate.

E: extraction of BSP by the liver.

Ca_{O_2}: arterial oxygen content.

$\Delta A\text{-}V_{O_2}$: arterial hepatic vein oxygen difference.

(*) Same figures for S_{BSP} in both runs were used in these cases because of technical difficulties.

ing "pure O_2" the means obtained in those cases were compared, showing that the difference: 8 ml/min was not significant and did not invalidate its inclusion in the present series (table II).

Carefully revising the falling plasma BSP concentration we have been able to see that in 2 patients (V. G. and M. D. C.), with plasma concentrations considerably lower than 1 mg %, the curves after an even

TABLE II (1)

	Cl_{BSP_p} ml/min		EHBF ml/min	EHBF ml/min°		$\dot{V}_{O_2}H$ ml/min°		A-V_{O_2} ml	
	1	2		°1	2	°1	2	°1	2
Mean	607.11	565.5	1702.9	1733.16	1728.16	84.67	95.56	4.62	5.14
$\bar{\delta}$	233.71	238.67	678.5	852.05	1004.1	33.47	43.54	0.245	0.678
$\delta_{\bar{x}}$	82.64	97.43	226.1	347.91	409.8	16.73	25.13	0.122	0.391
p	0.722		—			0.93		0.15	

(1) Symbols similar to Table 1 or

$\bar{\delta}$ = standard deviation of the population.

$\delta_{\bar{x}}$ = standard error of mean.

p = probability.

° = 1 and 2 designate consecutive determinations.

fall would suddenly become very steep. According to Sheila Sherlock et al. (5), at these plasma levels, the computation of SHBF would give falsely high figures. In two other of our cases (A. Z. and A. B.) the disappearance of BSP in plasma was the same above and below 1 mg % concentration, results which agree with those of Vetter et al. (8). Considering the patients in which the disappearance rate was estimated as even: in three of them (V. G., F. R. and J. B. H.) BSP extraction was computed with concentrations below 1 mg % while in 6 (A. Z., A. B., A. D. C., J. B. M., A. D. and J. N.) higher concentrations were used. Splanchnic oxygen consumption was 84.67 ml/min ($\bar{\delta}$ = 33,47 and $\delta_{\bar{x}}$ = 16.73) in four cases. In the duplicate estimation it reached 95,56 ml/min ($\bar{\delta}$ = 43.54 and $\delta_{\bar{x}}$ = 25.13) with a difference between means of 10.88 ml/min not significant (p = 0.93).

Mean oxygen extraction was 4.62 vol. % ($\bar{\delta}$ = 0.245 and $\delta_{\bar{x}}$ = 0.122)

breathing air while when it was measured breathing "pure O_2"; reached a value of 5.14 [difference which was not completely significant $p = 0.15$)].

Discussion

The method is based upon several important premises: 1) that BSP is cleared only at the splanchnic area; 2) that BSP clearance may be correctly calculated from the disappearance curve; 3) that extraction can be calculated without major error.

1) BSP is taken up by the liver and reticulo-endothelial system throughout the body. The liver contributes in more than 80 % to the uptake of injected BSP ([9]). When rather large amounts of this drug are injected, the total uptake is more representative of hepatic behaviour since the reticulo-endothelial system may be saturated and on the other hand its proportional participation is less significant ([5]).

2) The clearance of BSP and the S_{BSP} used in the computations are not seemingly objectionable taking into account our previous results ([7]) and those of other authors.

3) In relation to the value of the A-V difference of BSP, we must say that the brachial artery sample represents the blood flowing into the liver since almost the same amount of BSP may be found ([1]) in the hepatic artery, portal vein or peripheral vessels. Anyhow, the uptake of the drug by organs drained by portal blood would not change the results obtained. On the other hand, blood from the hepatic vein may not be absolutely representative of hepatic venous blood since its anatomical disposition forces to sample one or the other hepatic vein at one time. Studies by Bradley et al. ([1]) drawing blood from different hepatic veins and depths in the liver, seem to show that the differences are not important provided blood is not sampled too near the cava, since blood from this last vein may be aspirated and therefore contaminate the sample. In our cases the catheter was wedged and the sample taken slowly to obviate this source of error.

BSP recirculation through entero-hepatic system has been pointed out by Lorber and Shay ([13]) to be present in some patients 20 minutes after the injection. Our sampling is completed in that time so that we were not concerned with its occurrence in these patients.

4) Our results for hepatic blood-flow (table II) are comparable to those presented by Bradley et al. ([1]) * who obtained a mean figure of 1509.3 ml ($\overline{\delta} = 276.74$ and $\delta_{\overline{x}} = 57.70$), and Wade et al. ([14]) who found a mean of 1800 ml ($\overline{\delta} = 344.6$ and $\delta_{\overline{x}} = 154.1$). Both used the continuous infusion method. On the other hand, our results are reproducible. Two consecutive determinations in the same patients (table I) give a difference between means of 5 ml (not statistically significative) while Wade et al. ([14]) in two consecutive estimations of EHBF obtained a difference of 87.5 ml ($p = 0.74$).

Likewise, the results and differences between two estimations of splanchnic oxygen consumption and clearance of BSP (table II) are comparable to those obtained by Wade et al. ([14]).

* Statistical analysis made with the figures published by Bradley et al. (1) and Wade et al. (14).

SUMMARY

The estimation of EHBF is possible with a method which has the following characteristics.

1) A single injection of BSP is used which permits the saving of a rather expensive substance while eliminating the need of a cumbersome procedure like continuous infusion.

2) Rapidity of the procedure eliminates the problem of entero-hepatic circulation of BSP.

3) Body space of BSP may be estimated.

4) Systemic blood-flow and so called "central blood volume" may be measured concomitantly by the method of Stewart Hamilton.

5) The results obtained are reproducible and agree with those presented in the literature.

We acknowledge the technical assistance of Drs. Blanca Badano and Roberto González.

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THE GRAPHIC RECORDING OF THE RATE OF DRAINAGE OF BLOOD FROM THE VEINS OF THE LEGS

I. F. S. MACKAY AND C. C. GORDON

(The Department of Physiology University College of the West Indies, Jamaica, B. W. I.)

MACKAY (1) has reported an assessment of the rate of drainage of blood from the limbs by gravity. The method used did not provide a graphic record and the results were calculated on the basis of a volume drained as measured by plethysmography or by weight, measured with a balancing table technique.

The purpose of the study reported here is to describe a simple technique which graphically records the rate at which blood will drain by gravity from the legs.

It is of physiological interest to know the rate of this drainage as it provides an indication of the resistance to outflow of blood from the limb and, in addition, it may be useful for studying those clinical problems where there is an obstruction to the venous return such as in pregnancy, heart failure, etc.

This is a short preliminary report describing the method.

APPARATUS

The apparatus consists of a tilting table on which the subject lies supine and is firmly held in position by means of shoulder supports above and a bicycle saddle below [Mackay (2)]. The leg is placed in an air plethysmograph. Volume records are made by means of float chambers with frontal writing points recording on smoked paper. The plethysmograph is supported by elastic strapping so that the limb will not move in or out.

PROCEDURE

The recording of the rate of venous drainage from the legs

1) The body is tilted into the head-up position 52° from the hori-

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zontal (Filling Angle) for a period of one minute. This is more than sufficient time for the maximum filling of the limb.

2) At the end of the filling period, the body is steadily tilted in 2 to 3 seconds into the head-down position at varying angles from the horizontal (Drainage Angle). The time of the tilting is recorded by marking the points of commencement and completion of the tilting. It was found that the simplest way to carry out this tilting was to do it manually, and with practice this could be done in a constant fashion both in rate and time of tilt (2 to 3 seconds).

3) A record is made of the limb volume changes that occur during and following this manoeuvre.

Artifact volume records were made by completely occluding the circulation of the limb with a blood pressure cuff at 320 mm Hg (see figs. 1 and 2). After an artifact volume was recorded, it was subtracted from the total drainage volume record and a net volume curve of the limb was constructed, and from this the venous drainage rate was calculated.

In figure 1 (a and b) are presented typical tracings of artifact and total volume changes obtained by this procedure. The lower dotted curves represent the finally constructed net limb volume change from which is calculated the venous drainage rate.

The venous drainage rate is calculated from the "net volume" curve. The initial angle is drawn from the point where the tilting has been completed. It was felt that too many factors are involved during the tilting and that it was best to pay attention only to the rate of drainage that occurs when the tilting process has been completed.

As venous drainage will be the first event causing a change in limb volume, it was considered reasonable to use the term "venous" because the *initial* angle of the outflow was measured.

For the purpose of clinical investigations the drainage angle of 40° from the horizontal in the head-down position used in the above experiments might be too steep and uncomfortable, particularly for cardiac patients (fig. 1 a). So, it was decided to examine the drainage rate at an angle of 10° tilt. Quite satisfactory records can be made in normal subjects at this drainage angle (see fig. 1 b).

Different factors will play a part in creating an impedence to venous drainage at these two extremes of drainage angle, 10° and 40°. At an angle of 40° the height of the column of blood from the foot to the right atrium will be approximately 75 cm. While, when the body is tilted 10° head-down the height of the column will be only 15 cm. So that, at 40° the resistance caused by central venous pressure will play a less important role than at 10°. It is probable that at a drainage angle of 40° the major factor determining the rate of venous drainage would be the resistance to outflow in the veins themselves.

The question uppermost in our minds at this point was — "Is this technique going to provide an indication of resistance to venous drainage where there is a rise in central venous pressure?". This was examined by causing a physiological resistance to venous drainage by getting the subject to blow against a mercury column at various pressures. This positive valsalva was commenced 10 seconds prior to tilting. The effect

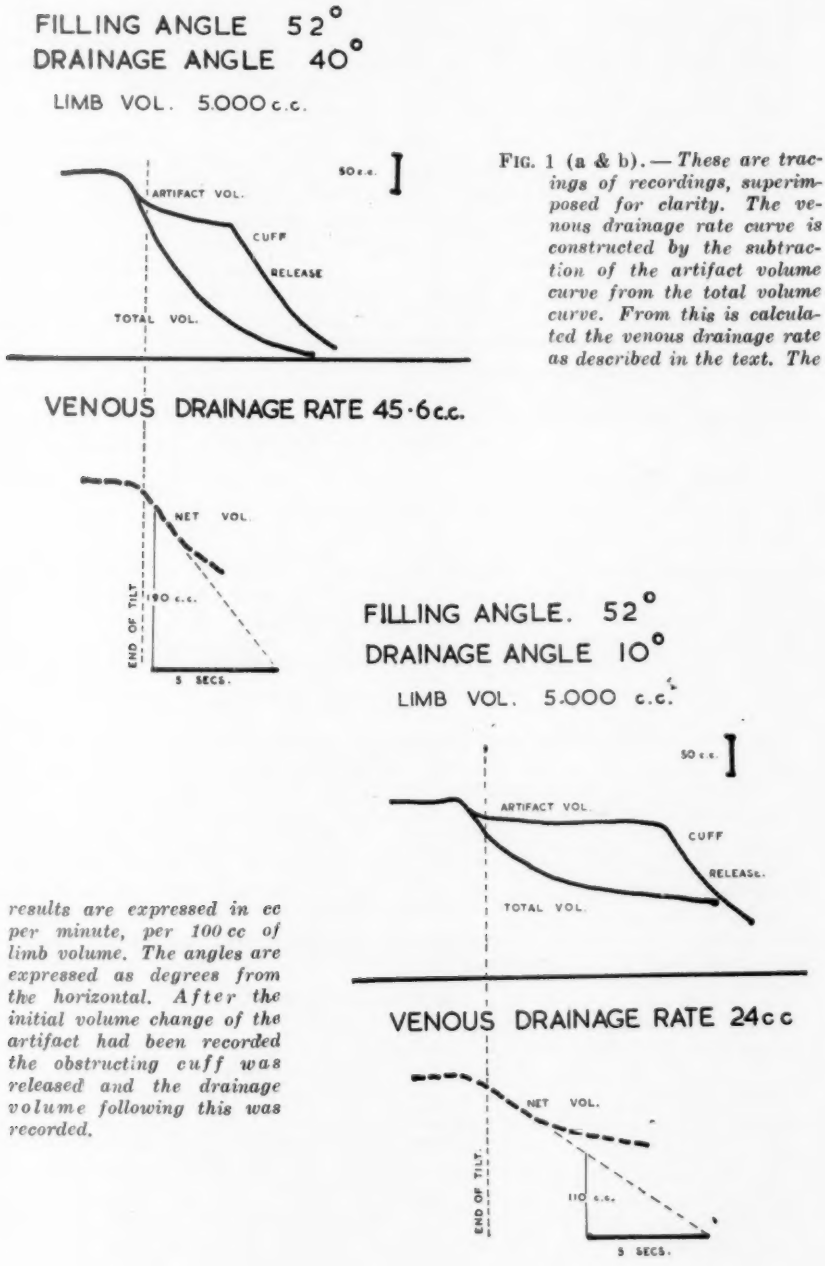

FIG. 1 (a & b). — *These are tracings of recordings, superimposed for clarity. The venous drainage rate curve is constructed by the subtraction of the artifact volume curve from the total volume curve. From this is calculated the venous drainage rate as described in the text. The results are expressed in cc per minute, per 100 cc of limb volume. The angles are expressed as degrees from the horizontal. After the initial volume change of the artifact had been recorded the obstructing cuff was released and the drainage volume following this was recorded.*

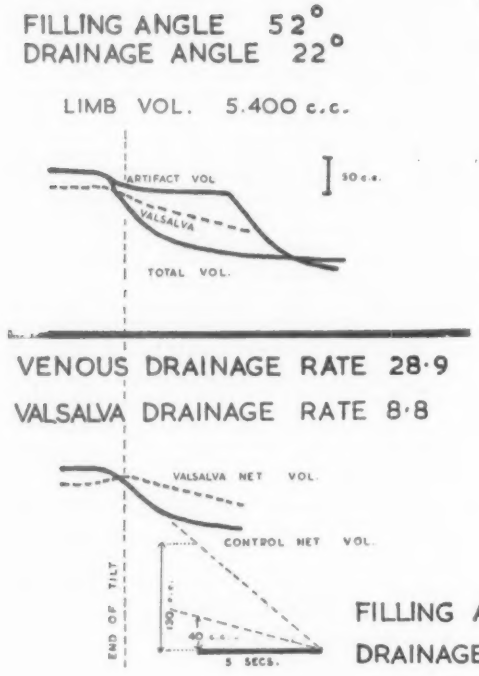

FIG. 2 (a & b). — *These figures demonstrate the influence of a positive valsalva manoeuvre upon the venous drainage rate. The positive valsalva was obtained by blowing a mercury manometer to a height of approximately 60 mm for ten seconds prior to the tilting and maintaining this pressure during and following the tilting procedure.*

of such a manoeuvre is shown in figure 2 (a and b) where it can be seen that the venous drainage is impeded while a positive intrapulmonary pressure of 60 mm Hg is being maintained. Where the drainage angle was 10° the outflow of blood from the legs was completely obstructed by this positive valsalva manoeuvre. At a drainage angle of 40° it was markedly reduced.

The degree of obstruction that might be caused by the elastic cuff of the plethysmograph was examined by observing the smallest venous occlusion pressure that might cause a rise in limb volume with the limb in a horizontal position. This pressure was not allowed to be larger than 10 mm Hg.

Experience has shown us that in doing studies on the peripheral vascular system on natives of a subtropical climate such as the West Indies, it is not advisable to use a room temperature of approximately 20° C, which is used for studies in more temperate zones. Therefore, in these experiments the ambient temperature was used which varied between 25 and 29° C during the series of experiments, but remained constant during each individual experiment.

Discussion

The results reported by Mackay (1) for the rate of drainage recorded in the first second were as follows:

Forearm and hand	178	cc/100 cc/minute
Legs	82.5	cc/100 cc/minute
Hand	198	cc/100 cc/minute

These earlier results were approximate but they indicated the lack of resistance to venous outflow that exists in the limbs. The results recorded here for the venous drainage rate of the leg do not demonstrate so large a drainage rate. One of the explanations for this discrepancy must be that in the earlier records the calculations were based on measurements that were made during the act of tilting, whereas the measurements recorded here were calculated from the time that the tilting was completed.

These results indicate again the ease with which blood can be drained from the limbs by gravity and the lack of resistance that must exist to venous outflow from the limbs.

It should be added that when it was decided to examine the possibility of a graphic method for recording venous drainage, the first approach made was to fill the limb and then apply and occluding cuff at 300 mm Hg. The subject was tilted into a head-down position. After 15 seconds, a record was made of the drainage rate following the release of the obstructing cuff. It was decided that this procedure did not provide very significant information as the drainage rate under these circumstances was recorded after the veins above the cuff had drained, which meant that the subsequent drainage rate recorded might not reflect changes in central venous pressure. Such records of the limb volume changes following cuff release can be seen in figures 1 and 2. The

drainage rates recorded under these conditions are always more rapid. The technique where no obstructing cuff is employed described here provides information of the drainage rate without any interference or separation of the limb veins from the veins leading to the heart.

SUMMARY

A simple technique is described for the graphic recording of the rate of venous drainage from the legs by gravity. The procedure involves the use of a tilting table and a limb plethysmograph. The initial angle following the completion of the tilting was recorded and from this the venous drainage rate was calculated. The results indicate the freedom with which venous drainage occurs and the lack of resistance that exists to the venous outflow from a limb.

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PROCEEDINGS OF THE ARGENTINE SOCIETY OF BIOLOGY

September, 5th, 1957

Distribution of sulphydryles and disulphide in the neurohypophysis and hypothalamus of the toad. by A. LASANSKY AND D. D. SABATINI. *Facultad de Ciencias Médicas, Instituto de Anatomía General y Embriología, Buenos Aires.*

Bennett's technique for detection of sulphydryles was applied to toad hypothalamus and neurohypophysis, previously treated by sodium glycolate.

In the neurohypophysis the substance giving a positive reaction with this method, was distributed in coincidence with the Gomori positive substance. In preoptic nucleus a divergence was found between these reactions.

Action of Coxsackie Virus group B type 4 on human lipoma "in vivo" and "in vitro". by E. S. DE LUSTIG, A. TEYSSIÉ AND D. UGAZIO. *Instituto Nacional de Microbiología, Av. Vélez Sársfield 563, Buenos Aires, e Instituto Nacional de Oncología "Angel H. Roffo", Av. San Martín 5481, Buenos Aires.*

Human lipomas obtained from biopsies were cultivated *in vitro* and on the 10th day of culture infected with Coxsackie Virus group B type 4. We studied the action of virus on adipose and fibroblast infected cells.

The cytopathogenic effect is not generalized to all migrated cells but only to a part and the mitotic cells are particularly affected.

The nucleus is more damaged than the cytoplasm: the nucleus is often deformed, eccentric, vacuolized; the cromosomes are desorganized, fragmented, clumped.

Six patients were treated with Coxsackie B_4 directly in the tumoral mass of lipoma: one lipoma disappeared completely at the 3th month after the 2nd. inoculation.

Two isolated lipomas regressed remarkably.

Two patients with multiples lipomas showed nodule regression and softening when inoculated directly but not those surrounding them.

Neutralizing serum previously inoculated prevented the effect of the virus in the sixth patient.

No clinical reaction which can be attributed to the virus has been observed: only a slight fever in the first patient who was cured definitively.

Histologically, the lipomas have been more susceptible than fibrolipomas.

October, 3rd, 1957

Adrenal cortex and medulla and resistance to hypoglycemic sulphonamides. by B. A. HOUSSAY, J. C. PENHOS AND J. APELBAUM. *Instituto de Biología y Medicina Experimental, Costa Rica 4185, Buenos Aires.*

Adrenalectomized rats were 30 times more sensitive than normal animals to

the toxic effect of 2254 RP. Medullectomized animals were 3-4 times more sensitive than normals to this effect.

Adrenalectomized rats were 40 times more sensitive than normal rats to the toxic effect of 2259 RP. Medullectomized animals were 1.5-2 times more sensitive than normals to this effect.

Adrenaline and hydrocortisone have a protective effect, but this effect is much more intense when both substances are given associatedly.

The sensitivity of adrenalectomized animals shows to be due to the simultaneous absence of cortex and medulla, but the role of the cortex seems to be quantitatively more important.

Carbon dioxide fixation during glucose oxidation by yeast. by A. O. M. STOPPANI, S. L. S. DE FAVELUKES, L. CONCHES AND F. L. SACERDOTE. *Cátedra de Química Biológica, Facultad de Ciencias Médicas de Buenos Aires y Laboratorio de Metabolismo Celular, Comisión Nacional de la Energía Atómica.*

1) Baker's yeast *(S. cerevisiae)* incorporates radioactive carbon dioxide during the aerobic and anaerobic oxidation of glucose, and brewer's yeast *(S. carlsbergensis)* during glucose fermentation. 2) The anaerobic fixation of carbon dioxide by baker's yeast (under nitrogen atmosphere, or in the presence of antimycin A has the same or higher efficiency than the aerobic fixation. 3) After steady state fixation, the main reservoirs of C^{14} are aspartic and glutamic acids. Malic, tricarboxylic, fumaric, succinic, phosphoglyceric and phosphopyruvic acids, alanine and hexosephosphates become also radioactive. 4) In the absence of oxygen or in the presence of antimycin A, labelling of malic, fumaric, succinic and aspartic acids increases, whereas that of glutamic acid decreases. 5) Carbon dioxide is incorporated by synthesis of oxalacetate through carboxylation of phosphopyruvate. 6) The distribution of C^{14} in aspartic and glutamic acids is consistent with the operation of the citric acid cycle as the main oxidative pathway in baker's yeast.

PROCEEDINGS OF THE SOCIETY OF BIOLOGY
OF SANTIAGΛ (Chile)

August, 14th, 1956

Influence of glycogen content on the effect of 2.4-Dinitrophenol on the synthesis of p-Aminohippurate by rat liver slices.

by HERMANN NIEMAYER, CARMEN GONZÁLEZ, ENRIQUE FIGUEROA AND HAROLD H. COGHLAN, with the technical assistance of JASNA RADOJKOVIC. *Instituto de Química Fisiológica y Patológica, Universidad de Chile.*

The effect of 2.4-Dinitrophenol (DNP) on the oxygen consumption by rat liver slices incubated with or without substrates depends on the glycogen content of the tissue (Niemeyer and Figueroa, *Arch. Biochem. Biophys.*, 1955, *54*, 135). In addition, the effect of DNP on the synthesis of glycogen from hexoses in liver slices may be counteracted by increasing the initial level of glycogen content (Niemeyer et al., *Acta Physiol. latinoamer.*, 1956, *6*, 117). In this communication, the influence of glycogen content and exogenous glucidic substrates on the effect of DNP on the synthesis of p-Aminohippurate (PAH) by rat liver slices was investigated.

0.025 mM DNP inhibits PAH synthesis and the effect varies according to the tissue glycogen content: with initial glycogen of 0.1 % or less, the average inhibition was 52 ± 2.0 %; with more than 1 % of glycogen the inhibition was 29 ± 1.4 % ($t = 9.3$). Fructose-di-phosphate (F-di-P) and pyruvate, added to the incubation medium at a concentration 0.025 M, counteract the inhibitory effect of DNP in slices with low glycogen content.

At higher concentrations of DNP (0.1 mM), high glycogen content still protects PAH synthesis, F-di-P protects only if glycogen level is high and pyruvate exerts no significant protection.

The anaerobic inhibition of PAH synthesis is counteracted neither by high glycogen content nor by the addition of F-di-P and pyruvate.

Exogenous adenosinetriphosphate (ATP) does not protect against DNP. Evenmore, ATP by itself inhibits the synthesis of PAH.

Sympathetic contribution to the vagal cardiac stimulating effect.

by D. BENÍTEZ, B. HOLMGREN AND S. MIDDLETON. *Instituto de Fisiología, Universidad de Chile.*

Since the early experiments by Jourdan and Nowak (1934) who obtained cardiac acceleration by stimulating the vagal rootlets at the medulla or the cervical portion of the vagal trunk in the atropinized animal, several authors [Brouha, Cannon and Dill (1936); Hodes (1939); and Kabat (1940)] have apparently demonstrated the existence of cardiac stimulating effects mediated by the vagi.

Middleton, Middleton and Tohá (1949) considered this effect as possibly due to the presence of adrenergetic postganglionic elements in the heart connected with the vagi. As this effect did not disappear after the degeneration of the centrifugal vagal fibers by section above the nodose ganglion, and a previous and complete sympathectomy had not been performed, it was considered of importance to undertake an experimental revision of their results.

Experiments were performed in adult cats; some of which had suffered complete unilateral cervical sympathectomy and the excision of the stellate and 2 to 4 thoracic ganglia. Others, were submitted to section of the right vagal nerve above or below the nodose ganglion. Some cats had suffered both operations.

Isolated heart preparations with dissected vagi according to a modified Langendorff's technique were usually performed 7 days after the operation.

After vagotomy the electrical stimulation of the cervical portion of the nerve showed no effect whatsoever on the heart, either before or after perfusion with atropine. However, if the thoracic portion of the nerve was stimulated, a typical positive inotropic effect was observed, both before and after atropine. This effect did not disappear when tetraethylammonium bromide was added to the perfusion fluid (Tyrode solution).

If the experiment was done in sympathectomized animals, no cardiac stimulating effects could be obtained whether the vagi were intact or degenerated.

It is concluded that the cardiac stimulating effects obtained from the cervical or thoracic portions of the vagus nerve in the cat depend on postganglionic sympathetic fibers included in the vagal trunk from stellate and cervical ganglia.

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The effect of plasma of anemic rabbits on plasma and red cell iron turnover. By George Hodgson, Irma Eskuche and José Tohá. *Sección Biológica, Laboratorio de Física Nuclear, Universidad de Chile.*

Previous work (Hodgson, G., Tohá, J., *Blood.*, 1954, *299*, IX) has shown that repeated injections of plasma of bled rabbits produce reticulocytosis and an increase of red cell and Hb concentration in normal rabbits. Since the study of iron turnover presents a rapid objective method for determining the intensity of erythropoiesis (Huff et al., *J. clin. Invest.*, 1950, *29*, 1041), it was decided to investigate this aspect of iron metabolism in normal rabbits and in rabbits injected with normal, anemic (bled) and anemic (Phenylhydrazine) rabbit plasma. The dose of plasma was 20 ml intraperitoneally, twice a day for two days. Tracer doses of high specific activity $Fe^{59}Cl_3$ (previously incubated with rabbit plasma) were injected intravenously on the 3rd day. The proportion of plasma iron turnover per hour K(%/hr) was calculated from the plasma radioactivity half times: $K(\%/hr) = 0.693/T_{\frac{1}{2}}$.

The following values for K(%/hr) were obtained: Normal rabbits: 56.7 ± 2.9 (standard error); rabbits injected with normal rabbit plasma: 60.6 ± 3.68; anemic (bled) rabbit plasma: 104 ± 7.14; and anemic (phenylhydrazine) rabbit plasma: 117 ± 6.66.

The percentage of the dose of Fe^{59} appearing in the red cells at 48 hrs, was 80.5 ± 1.85 in the controls (normal plasma) and 91.6 ± 1.41 in the rabbits injected with anemic (bled) plasma.

It is concluded that the determination of iron turnover presents a useful and objective biologic test for further study of the factor (or factors) responsible for the activity of anemic rabbit plasma.

August, 28th, 1956

Synthesis of phosphocreatine by enzymes from heart and skeletal muscle. by OSVALDO CORI, RENÉ FRENKEL AND AÍDA TRAVERSO CORI. *Instituto de Fisiología, Escuela de Medicina y Departamento de Biología Química, Escuela de Química y Farmacia, Universidad de Chile.*

An enzyme can be extracted from rat skeletal muscle, which is able to synthesize phosphocreatine in the absence of added adenine nucleotides, if phosphoglycerylphosphate is generated from fructose diphosphate in the same tube. The system contains no chemically or spectrophotometrically detectable adenine nucleotides (Kalckar, *J. biol. Chem.*, 1917, *167*, 467).

Enzymes utilizing ATP do not inhibit phosphocreatine synthesis (hexokinase, ATP-ase) whereas they do inhibit its formation from ATP and creatinephosphokinase.

3-phosphoglyceric acid cannot replace fructose diphosphate, unless ADP is added. Enzymatically prepared and isolated phosphoglycerylphosphate promotes a mole synthesis of phosphocreatine.

The enzyme utilizing phosphoglycerylphosphate exhibits a pattern of ammonium sulphate solubility different from creatine phosphokinase, thus pointing to two separate enzymes.

Preparations obtained under identical circumstances from rat heart are unable to synthesize phosphocreatine from phosphoglycerylphosphate, if no adenine nucleotides are added. Creatine phosphokinase is present in these preparations.

Synthesis of phosphocreatine from phosphoglycerylphosphate without the mediation of adenine nucleotides is postulated, considering the present evidence and thermodynamic data (Ogston, *Physiol. Rev.*, 1948, *28*, 283).

Functional modifications of the cardiovector system in experimental hyper and hypothyroidism. by HERMAN VAN DEN BELD, ROBERTO DOUGLAS AND JAIME TALESNIK D. *Laboratorio de Fisiopatología, Escuela de Medicina, Universidad de Chile.*

Previous reports have shown that the thyroid hormone affects the basic cardiac rate by a direct action on the heart.

The present work supplies more evidence that hearts of hyper and hypothyroid rats and cats maintain characteristic tachycardia and bradycardia when isolated.

Studying the mechanism by which the thyroid hormone modifies the heart rate, it was found that:

1) The sinus rythm is significantly different in the hearts of the three groups studied, namely hypothyroid, normal and hyperthyroid animals.

2) The removal of the sinus pacemaker leads to a decrease heart rate, but the differences observed in the three experimental groups are maintained.

3) If the interventricular septum is sectioned, the heart rate decreases even more, yet the differences between ventricular rate of hypo and hyperthyroid animals persist.

4) Cardiac escape of infrasinus origin develops by blocking the auricular pacemaker with acetylcholine. The ventricular rythm thus obtained in the three groups has the same characteristics as that described above.

5) The thyroid hormone influence on the various segments of the cardio-vector

system is evident when new pacemakers are established. Thus the cardiac arrest that precedes the idioventricular rythm, is significantly greater in hearts from hypothyroid animals than in normal and hyperthyroid ones.

6) Thyroxine induced tachycardia persists long after hormonal treatment is discontinued, althogh calorigenesis has returned to its normal level. This observations emphasizes the importance of the thyroid hormone as one of the factors with a regulating action on the genesis impulses that determine the basic cardiac rate.

These findings and their possible relations to some cardiac disturbances frequently observed in myxedema and thyroxicosis are discussed.

September, 11th, 1956

Adaptation rate as a function of stretch of cutaneous mechano-receptive nerve endings. by W. R. LOEWENSTEIN. *Instituto de Fisiología, Universidad de Chile.*

The behaviour of a fast and slowly adapting mechano-receptor of the frog's skin was studied over an ample range of skin stretch. The functional properties of a slowly adapting mechano-receptive fibre of the skin are described. The fibre has the characteristics of a slowly adapting stretch receptor, namely a graded stationery discharge in reponse to steadily maintained stretch and a period of depressed activity following sudden release of stretch. The other mechano-receptor of the frog's skin, the well know touch ending is readily excited by stretch (Loewenstein, 1956). At a low range of skin stretch, the tactile unit behaves quite differently as compared with the cutaneous stretch fibre, giving an "on" and "of" discharge to respectively the application and the release of stretch. Both responses adapt rapidly to zero activity. Over a certain range of stretch the number of tactile impulses and the total duration of the "on" response increase logarithmically with stretch. Finally at high stretch the touch unit acquires the properties of a slowly adapting stretch receptor: in response to continuously sustained stretch it no longer adapts to zero frequency but presents a stationery discharge instead, and the "off" response is replaced by a period of inhibited activity.

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Mechanical component in adaptation of mechano-receptors. by W. R. LOEWENSTEIN. *Instituto de Fisiología, Universidad de Chile.*

A hypothesis is brought forward which considers a mechanical component in adaptation of mechano-receptors. Rapidly adapting receptors are assumed to have folded, slowly adapting receptors, unfolded membranes at resting length. Whether the receptors ending suffers a transient or continuous depolarization, i. e., whether the receptor adapts or not in response to a continually sustained mechanical stimulus, will depend in part on the ability of in-series elastic elements to maintain the nerve ending outstretched. Thus, as shown in preceeding paper, a tactile receptor, the mechano-receptor of rapid adaptation par excellence, when fully stretched may behave like a slowly adapting receptor which presents a stationery discharge.

October, 9th, 1956

Simple method for labeling thyroxine with I^{131}. by WOLFGAG HÜLSEN AND JOSÉ CALVO. *Instituto de Química Fisiológica y Patológica, Universidad de Chile.*

To label Thyroxine with I^{131} three different methods have been followed:

1) the chemical, 2) the biological, 3) by isotopic exchange. In the latter method the iodine substituted atoms of Thyroxine exchange or permute with the free iodine atoms of the solution in such a way that after a certain time some of the iodine at the Thyroxine molecule exhibits a fraction of the radioactivity that was previously in inorganic condition.

In all the methods proposed for isotopic exchange it is important that the I^{131} that exchanges, with or without carrier be in free condition and that the solution should possess an acid pH. The reaction conditions employed by various investigators differ with respect to contact time (from hours to minutes), temperature, the nature of the solvent in which the reaction takes place and the methods employed for the separation of the radioactive Thyroxine: recrystallization, chromatography and a selective extraction with an organic solvent.

In the method that we propose the Thyroxine is added to an aqueous solution containing a certain portion of KIO_3, the I^{131} tracer and some amount of HCl in order to maintain the pH around 1.0. The Thyroxine, that is dissolved in NaOH, is added dropwise and precipitates and after one or two redissolutions and precipitations the radioactive Thyroxine is sufficiently pure for further applications.

In the procedure followed it is possible to recover 85 % of the initial Thyroxine with approximately 50 % of the starting radioactivity.

The thyroxine so obtained was verified by chromatographyc analysis in Whatman Nº 1 filter paper, using different solvent mixtures and also through radiochromatography, 97 % of the radioactivity was recovered in the Thyroxine spot, the remaining radioactivity corresponding to the iodine.

Cytochrome system in Trichinella Spiralis. by MOISÉS AGOSIN. *Laboratorio de Parasitología, Universidad de Chile.*

October, 23rd, 1956

Calorigenic action of 2.4-Dinitrophenol in normal and adrenalectomized rats. by WILMA TOLEDO MARCHANT AND JAIME TALESNIK. *Laboratorio de Fisiopatología, Escuela de Medicina, Universidad de Chile.*

In previous papers it has been shown that thyroxine does not increase the oxygen consumption of the adrenalectomized rats unless an adequate supply of corticosteroids are given.

2.4-Dinitrophenol (DNP) was administrated to rats in order to establish whether corticosteroids are also required for this substance to exert its calorigenic action.

Various routes of administration of DNP were tried; a more sustained increase of oxygen consumption was shown by rats fed DNP included in an adequate form in their diet.

DNP increases oxygen consumption in adrenalectomized rats in a manner similar to that of normal animals. Moreover, adrenalectomized rats, in the last stages of adrenal failure, also responded with an increase of oxygen consumption to DNP.

Prolonged administration of DNP does not modify the weight of rat adrenals as thyroxine does.

It is concluded that treatment of rats with thyroxine increases the tissue demands for corticosteroids while increase in oxygen consumption by DNP does not have this effect.

Increase of the suplementary respiratory factor for red cells in the organs of thyroxine treated rats. BY J. M. CALVO, M. LOLAS AND R. URBA. *Instituto de Química Fisiológica y Patológica, Universidad de Chile.*

November, 13rd, 1956

Physiological and physiopathological mechanism of excretion of true endogenous creatine. by MITZY CANESSA AND SIGMUND FISHER. *Laboratorio de Función Renal, Cátedra de Medicina, Hospital Salvador, Universidad de Chile.*

Manitol clearance and true endogenous creatine clearance were studied in 18 periods in 7 normal subjects with glomerular filtration rates superior to 100 cc/min. The Cm/C cr ratio averages 0.992 ± 0.076 in the group, indicating that true endogenous creatine is excreted only by glomerules.

In patients with glomerular filtration rate between 50 and 100 cc/min, the rate Cm/C cr was 0.935 ± 0.377.

In subjects with Cm under 20.0 cc/min the rate was 1.123 ± 0.34.

The administration of large dosis of diodrast, depresses the true endogenous creatine clearance only in 2 of 9 patients studied; this event was related with a depressed renal plasma flow.

The graphical studies indicate that true endogenous creatine suffers only process of glomerular filtration and its clearance is dependent of tubular activity, except in certain patients with advanced renal failure.

Part played by the anterior pituitary gland in the adreno-cortical stimulating effect of thyroxine. by J. DE D. VIAL, D. RAMÍREZ AND J. TALESNIK. *Departamento de Anatomía, Escuela de Medicina, Universidad Católica y Departamento de Fisiopatología, Escuela de Medicina, Universidad de Chile.*

Thyroxine increases the oxygen consumption of hypophysectomized rats. The maximum increase in oxygen consumption expressed as percentage of the pre thyroxine treatment levels is similar in control and hypophysectomized animals. The response of hypophysectomized rats to thyroxine, injected twelve days after hypophysectomy, is similar to that of control rats and becomes apparent within twelve hours; on the other hand, hypophysectomized rats injected with thyroxine forty days after operation show no increase in oxygen consumption till after sixty hours.

The survival of both groups of thyroxine treated hypophysectomized rats, is short.

Hypophysectomized rats which had received an intraocular pituitary graft twelve days after operation, showed a rapid response to thyroxine (within twelve hours) injected forty days post-hypophysectomy. Survival in this group was significantly longer than in the nongrafted hypophysectomized rats.

The adrenals of hypophysectomized rats show progressive atrophy. Histologically, the reduction in size is more marked in the fascicular and reticular zones. Lipids, studied with polarized light microscope, tend to be distributed in two bands, in the fascicular and glomerular zones. In rats forty days after hypophysectomy birefringence is seen mainly in the fascicular zone.

The adrenals of hypophysectomized rats injected with thyroxine are similar to those of non injected hypophysectomized animals.

In the hypophysectomized rats with an intraocular pituitary graft atrophy of the adrenals is less marked. The lipids are more abundant and are distributed in

two clearly separated bands. Thyroxine treatment of these animals further increases gland weight but does not produce any further change in the histological picture.

The interactions between thyroxine and the anterior pituitary are discussed.

Conclusions: The adrenals of hypophysectomized rats continue to produce small quantities of corticosteroids.

Thyroxine is only able to modify the adrenals when the anterior pituitary is present.

The action of thyroxine on the anterior-pituitary is in part at least, a direct one, as shown by the experiments with intraocular pituitary transplants.

Influence of adrenalectomy and of some cortical hormones on the nucleic acids content of the liver, kidney and spleen of the rat. by R. GÓMEZ, J. SOLAR, N. PÉREZ AND M. PLAZA DE LOS REYES. *Instituto de Química Fisiológica y Patológica, Escuela de Medicina, Universidad de Chile.*

The influence of different hormonal substances on the nucleic acid metabolism has been subject of many recent studies (1, 3, 4) but till now the results are not definitive, because of the variability of the hormonal effects, obtained due to the different experimental conditions employed.

In this work we investigate in several groups of animals, the effect of adrenalectomy, and of adrenocortical hormone administration, in animals lacking adrenal function, on the nucleic acid content of the liver, kidney and spleen of the rat after 3 weeks of treatment.

The results obtained may be summarized as follows:

a) In adrenalectomized rats maintained with sodium chloride, without subtitution therapy, we found a significative increment on the content of liver desoxyribonucleic acid.

b) The administration of desoxycorticosterone and hydrocortisone independently or simultaneously, during 20 days, permits a significant fall in the content of ribonucleic acid of liver, spleen and kidney in the adrenalectomized rat.

c) In the same conditions as b), the desoxyribonucleic acid rises in the liver, and falls in the spleen, in both cases significatively. There is no change in the kidneys when two hormones are given simultaneously.

Considering Long's hypothesis (5) that hydrocortisone like steroids would produce a relative inhibition on the ribonucleic acid synthesis, due to a partial blockage on certain metabolic paths of glucose-6-phosphate, we can explain the fall on the ribonucleic acid content in the organs of hydrocortisone treated, adrenalectomized rats. The rise of desoxyribonucleic acid in the liver has been interpreted by supposing a greater independence of its biosynthetic pathways, of the hydrocortisone acetone.

The effect of desoxycorticosterone may be due to some real hydrocortisone like effect over nucleic acids metabolism or perhaps to the formation of a common metabolite, derived from desoxycorticosterone as well as from hydrocortisone, in adrenalectomized rats.

The lack of effect of both hormones on kidney desoxyribonucleic acid may be explained by the slow turnover of this substance in this organ.

The fall of spleen desoxyribonucleic acid content could be due to the lysis of lymphoid elements.

The rise in the liver desoxyribonucleic acid content after adrenalectomy has been interpreted supposing that suppresion of adrenal hormones could unmask the effect of other hormonal substances, normally acting in dynamic equilibrium with the former.

The fact that DOC or F administration does not correct the effects of adrenalectomy on liver desoxyribonucleic acid content is probably explained because these

hormonal treatments don't replace in a really physiological manner the quantity, or delivery rhythm, of the normal secretory pattern of the rat adrenal cortex.

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November, 27th, 1956

Thyroxine increase and 2.4-Dinitrophenol inhibition of aerobacter aerogenes growth. by JOSÉ CALVO AND RUTH URBÁ. *Instituto de Química Fisiológica y Patológica, Universidad de Chile.*

Dl Thyroxine in concentration over 5.0×10^{-5} stimulates the growth of Aerobacter Aerogenes when the culture medium contains, besides inorganic salts, glucose and certain amount of yeast extract as N source. In the same culture conditions, excepting the yeast extract, Thyroxine, at is well known, induces Aerobacter to increase the O_2 consumption (Weinfan and Marx, *J. biol. Chem.*, 1955, *214*, 441).

The growth of the Aerobacter was followed by turbidimetric observations at predetermined intervals and in 12 experiments after 24 hrs, and it was seen that this growth was 17 to 70 % when Thyroxine was present in the culture than when not.

Keeping in view the contingency that the turbicimetric increase might be the consequence of thyroxine precipitation in a continuous acid producing medium, germs were dried out and weighed before and after a pH 2.0 n.Butanol extraction, this solvent being known to disolve Thyroxine. It was found that the germs from the cultures containing Thyroxine, were heavier than those from control cultures.

In other experiments desoxiribonucleic acid was estimated in aliquots obtained from cultures with and without Thyroxine. Without exception the first ones were richer in the desoxiribonucleic acid than latter ones.

When the culture medium was deprived of glucose the observed growth differences disappeared notwithstanding the fact some proliferation persisted out at a lower level than in the control glucose cultures.

When Thyroxine was replaced by 3.5-Diidothyroxine or 3.5-Diidotirosine, Thyrosine or Iodide in equivalent amounts with respect to N content in the first three compounds or in the last, no growth effect was observed.

2.4-Dinitriphenol in a range between 1.25 and 5.0×10^{-5} inhibited the bacterial growth under the same culture conditions that were employed for Thyroxine. In 3 experiments the inhibitions was between 15 and 23 %.

The metabolism of thyroxine in aerobacter aerogenes studied with paper chromatography. by JOSÉ M. CALVO, JESÚS HERRERA AND LUZ BASCUR. *Instituto de Química Fisiológica y Patológica, Escuela de Medicina, Universidad de Chile.*

With unidimensional chromatographic analysis it is possible to resolve in separate spots, mixtures of Iodide, Thyroxine and related compounds.

Theoretically this method permits the following of the structural changes undergone by Thyroxine when this compound is acting upon Aerobacter Aerogenes

and producing a stimulus, according to culture conditions, upon respiration or growth.

In the actual experiments the germs were separated from the fluid medium by high speed centrifugation and the Thyroxine and prospective degradation compounds were extracted from both phases by using proper amounts of n.Butanol at pH 2.0.

The butanol extraction was repeated upon the germs until there was no more iodine detectable in the solvent as determinated by a sensitive test.

The butanol extraction finished, the mass of germs was washed out with saline solution and dissolved after a brief attack with 5 % NaOH or fully digested by exposure to Trypsin followed with Papain. Both germ hydrolysates were again extracted with butanol acid and these concentrated and examined for iodinated compounds.

The n.Butanol extracts obtained from culture fluid and germs were concentrated under reduced pressures at a low temperature and chromatographed. For chromatogram development n.Butanol-acetic acid-water (80:20; 100), solvent was employed and the iodine containing spots were detected after treatment with the catalytic ceric-arsenious reagent, that permits the discovery of Iodide, Thyroxine and allied compounds in amounts indicated below 1 μgm.

As no iodine spot other than of Thyroxine was detected in the chromatograms, and from the fact that no iodine was left in the germs mass after repeated n.Butanol extraction, the conclusion was drawn that Thyroxine keeps its chemical integrity during its metabolic effects upon the Aerobacter Aerogenes.

December, 11th, 1956

Evoked potentials by photic stimulation in the optical cortex of the cat. by F. ORREGO, O. GUTIÉRREZ, M. PALESTINI AND M. BORLONE. *Centro de Investigaciones Psiquiátricas, Universidad de Chile.*

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metro	m	m	milisegundo	ms	msec
centímetro	cm	cm	litro	l	l
milímetro	mm	mm	centímetro cúbico	cm^3	cc
micrón	μ	μ	mililitro	ml	ml
milimicrón	mμ	mμ	kilogramo	kg	kg
Angström	o A	o A	gramo	g	gm
microgramo	μg	μg	miligramo	mg	mg
gama	γ	γ	Miliequivalente	mEq	mEq
hora	h	hr	Curie	C	C
minuto	m	mín	Milicurie	MC	MC
segundo	s	sec	Microcurie	μC	μC
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